(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 159 286 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
03.03.2010 Bulletin 2010/09

(51) Int Cl.:
C12N 15/11 *(2006.01)* ***A61K 31/7088*** *(2006.01)*

(21) Application number: **08105194.8**

(22) Date of filing: **01.09.2008**

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MT NL NO PL PT RO SE SI SK TR
Designated Extension States:
AL BA MK RS

(71) Applicant: **CONSIGLIO NAZIONALE DELLE RICERCHE**
00185 Roma (IT)

(72) Inventors:
- **de Franciscis, Vittorio**
  80134 Naples (IT)
- **Cerchia, Laura**
  80122 Naples (IT)
- **Condorelli, Gerolama**
  80121 Naples (IT)

(74) Representative: **Capasso, Olga et al**
De Simone & Partners S.p.A.
Via Vincenzo Bellini, 20
00198 Roma (IT)

(54) **Method for obtaining oligonucleotide aptamers and uses thereof**

(57) The present invention relates to a method for obtaining nucleic acid aptamers that bind to cancer cell-surface epitopes, to the aptamers generated using this method and their use for therapeutic, diagnostic and prognostic purposes.

EP 2 159 286 A1

Description

FIELD OF THE INVENTION

[0001] The present invention relates to a method for obtaining nucleic acid aptamers that bind to cancer cell-surface epitopes, to the aptamers generated using this method and their use for diagnostic, prognostic and therapeutic purposes, including drug delivery.

BACKGROUND OF THE INVENTION

[0002] The hope of success of therapeutic interventions in cancer largely relies on the possibility to distinguish, with high accuracy, even closely-related tumor types. Indeed, the identification of tumor specific signatures has been a major challenge of the last ten years to predict the responsiveness to a given therapeutic plan and to reduce the impact of side effects to be expected if unresponsive oncologic patients are being treated.

[0003] The SELEX technique refers to Systematic Evolution of Ligands by EXponential enrichment. Single-stranded oligonucleotides have the diversity characteristic both in molecular structure and function, thus, a random library of single oligonucleotides is synthesized for binding to a target protein on the membrane. The oligonucleotides bound non-specifically are washed away and the oligonucleotides bound specifically were eluted in denatured condition and collected. The oligonucleotides are amplified by PCR for further selection. The high affinity oligonucleotides, namely aptamers that have high affinity with the target proteins, can be selected from the initial library through amplification and selection over many cycles. In 1990, Tuerk and Gold selected Aptamers of T4 RNA polymerase by SELEX (Tuerk C and Gold L. 1990). Subsequently, Ellington and Szostak showed great interests in the application of aptamers in scientific research and production. Aptamers soon become a valuable research tool and show great application prospected in the fundamental research, drug selection and clinical diagnosis and therapy (Ellington and Szostak, 1990). At present, many kinds of aptamers have come into clinical test phase. For example, drugs for curing thrombus and inhibiting endometrium hyperplasic and angiogenesis (Green LS et al., 1995, Tasset DM, et al., 1997, Ruckman J et al., 1998).

[0004] An innovative aspect of the aptamers is their use in "target identification/validation" to identify various cell surface targets of a specific cellular state.

[0005] The patent US 5,580,737 discloses a method for identifying nucleic acid ligands to a target molecule comprising contacting a mixture of nucleic acid with the target molecule, allowing the partitioning of increased affinity nucleic acid and then, contacting the increased affinity nucleic acid with non-target molecule. In particular ligand to theophylline and caffeine are described.

[0006] The patent application WO 2007/142713 provides a method for obtaining a probe specific for extracellular or cell-surface markers comprising several cycles of positive selection steps on a target cell followed by a step of counter-selection on a control cell. This method allows the selection of only a limited number of aptamers and only further to a high number of selection and/or counter-selection cycles. In addition, the selected aptamers display low cell specificity and are able to discriminate between cells of distant tumor types only (T-cell versus B cell lymphoma or small lung cancer cell versus large cell lung cancer, two cancer types of different origin).

[0007] Therefore, there is the need to provide a simplified method for obtaining aptamers comprising fewer cycles and resulting in aptamers with high specificity, even able to discriminate between different cells of the same tumor type, possessing different phenotypes (different resistance to a given physical or chemical therapeutic drug, different tumor mass growth properties, different ability to metastasize and different malignancy).

[0008] The authors of the present invention have already generated specific aptamers for the human receptor tyrosine kinase, Ret (Cerchia et al., 2005), however they cannot be used to solve the problem of the invention.

[0009] The present invention discloses a simplified method to generate nucleic acid-based aptamers that bind to cancer cell-surface epitopes as unique tools to identify a surface molecular signature of cancer cells and thus permits to generate a small panel of high specific ligands capable of distinguish between even two closely related cell types. This approach, based on the use of living cells as target for the aptamers selection (whole-cell SELEX), allows selecting aptamers in a physiological context, and, most importantly, can be done without prior knowledge of the target molecules. The methods include much fewer steps than prior art methods.

[0010] The nucleic acid-based aptamers of the invention are able to discriminate between malignant and non malignant cell phenotype. The aptamers can also discriminate two different phenotypes within the same tumor cell type as for example, the resistance to a given physical or chemical therapeutic drug, the growth properties of the tumor mass, the ability to metastasize and the malignancy. The panel of aptamer molecules obtained and obtainable with the method of the present invention represent an innovative tool to detect cell surface specific epitopes as a signature of cancer cells in terms of tumor type, malignancy, therapeutic response, metastatic potential, proliferation and apoptotic rate. The panel of aptamer molecules obtained and obtainable with the method of the present invention represent an innovative tool to specifically target cancer cell with given surface specific epitopes in terms of tumor type, malignancy, therapeutic

response, metastatic potential, proliferation and apoptotic rate.

SUMMARY OF INVENTION

[0011] Two types of human solid tumors were used as model systems, malignant glioma and non small cell lung carcinoma (NSCLC). Cultured human cancer cells that have close genetic background and only differ for their malignancy and/or therapeutic response were used as targets of the SELEX procedure. By coupling the Differential SELEX protocol to cancer cell lines, the authors were able to isolate different aptamers that are specific for targets present on the tumor cell type used (case 1: glioma; case 2: NSCLC) and absent on any other cancer type tested. Further, the authors of the present invention demonstrate that a small subset of aptamers is sufficient to distinguish two different cell lines of the same tumor type, but with different growth and therapeutic sensitivity (case 1 tumorigenic *versus* non-tumorigenic; case 2: TRAIL resistance *versus* sensitivity). Further, some of the aptamers have biological activity on the target cells.

[0012] Therefore it is an object of the present invention a method for selecting a nucleic acid aptamer specific for a protein selectively expressed on the cell surface of target cells comprising the steps of:

a) incubating a collection of synthetic nucleic acid oligomers with control cells, allowing oligomers to bind to them;
b) recovering a first set of unbound nucleic acid oligomers;
c) incubating the first set of unbound nucleic acid oligomers with target cells, allowing the first set of unbound nucleic acid oligomers to bind to them;
d) recovering nucleic acid oligomers bound to target cells;
e) amplifying and sequencing the nucleic acid oligomers bound to target cells.

[0013] Preferably, the first set of unbound nucleic acid oligomers recovered in step b) is incubated with the control cells and a second set of unbound nucleic acid oligomers recovered in step b) is further processed as indicated in steps c), d) and e).

[0014] Still preferably, the collection of synthetic nucleic acid oligomers is a synthetic library.

[0015] More preferably, the synthetic nucleic acid oligomers are labelled. Yet preferably the nucleic acid oligomers are oligoribonucleotides or modified RNA-se resistant oligoribonucleotides.

[0016] In a particular embodiment the target cell is a tumor cell and the control cell is a tumor cell of the same cell type as the target cell but having a different phenotype. Preferably, the tumor cell is a glioma cell or a NSCLC cell.

[0017] Still preferably, the phenotype is selected from the group of: resistance to a given physical or chemical therapeutic drug, tumor mass growth properties, apoptosis, ability to metastasize or malignancy, drug treated tumor cell.

[0018] It is a further object of the invention a nucleic acid aptamer obtainable according to the method of the invention. Preferably, the nucleic acid aptamer is for medical use. More preferably the nucleic acid aptamer is for the treatment of a tumor, also as targeting component for biocomplexes with nano particles or siRNAs. Still preferably the nucleic acid aptamer is for the diagnosis of a tumor and/or the follow-up of a therapy, also for molecular imaging. More preferably the nucleic acid aptamer is for predicting a therapeutic response of a drug for a tumor.

[0019] Preferably the tumor is a glioma or a NSCLC.

[0020] Still preferably, the nucleic acid aptamer is for the detection of a target cell.

[0021] Preferably, the target cell is a tumor cell. More preferably, the tumor cell is a glioma cell or a NSCLC cell.

[0022] More preferably, the nucleic acid aptamer has a sequence selected from the group of: SEQ ID No.3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104 or 105.

[0023] It is a further object of the invention a pharmaceutical composition comprising at least one nucleic acid aptamer of the invention and suitable excipients and/or diluents and/or carrier.

[0024] The present invention shall be disclosed in detail in the following description also by means of non limiting examples referring to the following figures.

Figure 1. Schematic Protocol for the Selection of cancer cell-specific aptamers. A pool of 2'F-Py RNAs was incubated with poorly tumorigenic T98G cells (Counterselection). Unbound sequences in the supernatant were recovered and incubated with tumorigenic U87MG cells for the selection step (Selection). Unbound sequences were discarded by several washings and bound sequences were recovered by total RNA extraction. Sequences enriched by the selection step were amplified by RT-PCR and in vitro transcription before a new cycle of selection. The same protocol has been used in the second example using the NSCLC cell line Calu1, for selection and the cells H460 for counterselection.

Figure 2. Evolution monitoring of the whole-living cells SELEX. (A) Increase in the selection stringency during

the SELEX protocol against Glioma (upper panel) and NSCLC (lower panel) . (B) Estimation of the pool complexity during each round of SELEX by RFLP. [^{32}P] 5'-end-labeled double-stranded DNAs corresponding to the population of candidates from each of the indicated rounds (starting pool is indicated as 0, rounds analysed are: 1, 5, 11, 12, 13 and 14 for the selection on glioma, left panel, and 3, 5, 6, 10, 12, 13 and 14 for the selection on NSCLC, right panel) were digested with a combination of RsaI, AluI, HaeIII, HhaI endonucleases and analyzed by electrophoresis on a 6% denaturing polyacrylamide gel. Enrichment of the nucleic acid pools is assessed as the enrichment of specific digestion fragments within the random population that are visualised as bands in lanes 12, 13, 14 (glioma) and lanes 10, 12, 13, 14 (NSCLC).

Figure 3. Binding analyses of the pool after 14 rounds of selection on glioma cells. The pool after 14 rounds of selection (named G14) or the starting pool (named G0) were 5'- [^{32}P]-labeled and incubated at increasing concentrations on the indicated cell lines.

Figure 4. Binding analyses of the pool after 14 rounds of selection on NSCLC. The pool after 14 rounds of selection (named L14) or the starting pool (named L0) were 5'- [^{32}P]-labeled and incubated at 500 nM on the indicated cell lines.

Figure 5. Alignment of sequences obtained from Whole-cell SELEX on glioma. 71 sequences (codified as B14, B10, C16, C23, ect..) obtained from the selection were aligned and analyzed using the BIOEdit sequence software. Out of 71 sequences obtained, 60 sequences are different. The sequences are reported as DNA and for simplicity, fixed-primer sequence at 5' and 3' extremities are removed.

Figure 6. Analysis of individual sequences similarity. Dendogram (obtained by using DNASIS software version 2.1) for visual classification of similarity among 71 individual sequences cloned after 14 rounds of selection. Aptamers that share sequence similarity are grouped in families (boxed); sequences found more than once are labeled with the asterisk.

Figure 7. Alignment of sequences obtained from Whole-cell SELEX on NSCLC. 55 sequences (codified as AL1, BL2, CL1 ect..) obtained from the selection were aligned and analyzed as described in the legend to Figure 5. Out of 55 sequences, 43 are different. The sequences are reported as DNA and for simplicity, fixed-primer sequence at 5' and 3' extremities are removed.

Figure 8. Dendogram for visual classification of similarity among the 55 individual sequences cloned after the selection on NSCLC cells.

Figure 9. First screening for binding properties of sequences obtained from Whole-cell SELEX on glioma cell lines. The indicated aptamers or the starting pool (G0) were 5'- [^{32}P]-labelled and incubated in the same condition at 500 nM on U87MG cells. The results are expressed relative to the background binding detected with the starting pool.

Figure 10. Comparison of a secondary structure prediction for C13, A5, D9 and A9 aptamers. Predicted secondary structures for C13, A5, D9 and A9 aptamers (with fixed-primer sequence at extremities). Structures were predicted using MFOLD software version 3.1 (available at http://www.bioinfo.rpi.edu/applications/mfold/) (Zuker, 2003).

Figure 11. Binding analyses of best sequences to glioma cell lines. The indicated aptamers or the starting pool (G0) were 5'- [^{32}P]-labeled and incubated in the same condition at 50 nM on the indicated glioma cell lines. The results are expressed relative to the background binding detected with the starting pool. The binding capacity of the aptamers to the cells is reported: high binding (more than four folds) is indicated as "++", middle binding (between two and four folds) is indicated as "+" and no binding (less than two folds) is indicated as "-". The tumorigenic potential in nude mice is indicated on the basis of the time of appearance of tumour and the tumour growth rate as previously reported (Ishii N *et al* (1999); Nishikawa R *et al* (1994); Pallini R *et al* (2006): high tumorigenicity is indicated as "++"; middle tumorigenicity is indicated as "+" and no tumorigenicity is indicated as "-").

Figure 12. Biological activity of selected aptamers. U87MG cells were serum starved for 2 hs and either left untreated or treated for 1h with 200 nM of the indicated RNA aptamer or the starting RNA pool (G0). (A) Cell lysates were immunoblotted with anti-pErk antibodies and then the filters were stripped and reprobed with anti-Erk antibodies to confirm equal loading. Quantitations are done on the sum of the two Erk-specific enhanced chemiluminescence bands of 44 and 42 kDa. (B) Cell lysates were immunoblotted with anti-pAkt, anti-Akt, anti-PDK1 and anti-pPDK1 antibodies. The filter were stripped and reprobed with anti-αtubulin antibodies to confirm equal loading. In A and B, intensity of bands have been calculated using the NIH Image Program on at least two different expositions to assure the linearity of each acquisition. Fold values are expressed relative to the reference points, arbitrarily set to 1 (labelled with asterisk). "C" indicates mock-treated cells.

Figure 13. Time-course experiment of the best inhibitors. Serum starved U87MG cells were either left untreated or treated with 200 nM of the indicated RNA aptamers or G0 for the indicated incubation times. (A) Cell lysates were immunoblotted with anti-pcyclin D1 and cyclin D1 antibodies.To confirm equal loading the filters were stripped and reprobed with anti-αtubulin antibodies. (B) Cell lysates were immunoblotted with anti-pErk antibodies and the filters were stripped and reprobed with anti-Erk antibodies. In A and B, quantitation and relative abundances are expressed

relative to controls, arbitrarily set to 1 (as reported in legend to Fig. 12); "C" indicates mock-treated cells. Plots of fold values corresponding to the cyclin D1 expression and to Erk activity are reported for each lane of immunoblotting shown in A and in B, respectively.

METHODS

Cell culture and immunoblotting

[0025] Human glioma U87MG (American Type Culture Collection, ATCC no. HTB-14) and T98G (ATCC no. CRL-1690), U251MG and TB10 (kindly provided by A. Porcellini) cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (Invitrogen, Carlsbad, CA). Human glioma, LN-18 (ATCC no. CRL-2610), LN-229 (ATCC no. CRL-2611) were grown in Advanced DMEM supplemented with 2 mM L-glutamine, 10% fetal bovine serum (Invitrogen, Carlsbad, CA). U87MG.ΔEGFR (Nishikawa R *et al.*,1994), a U87MG-derived cell line expressing a truncated mutant EGFR receptor due to an in-frame deletion of exons 2-7 from the extracellular domain (ΔEGFR or de 2-7 EGFR), were grown in DMEM supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 500 μg/ml gentamycin (Invitrogen, Carlsbad, CA). Growth conditions for cell lines used were previously reported: human neuroblastoma SH-SY5Y and SK-N-BE cells (Esposito CL et al., 2008), human breast MCF7 and SKBR3 cells (Buckley MF et al., 1993), human NSCLC H460, Calu1, A459 and A549 cells (Zanca et al., 2008) and NIH3T3 cells (Cerchia et al., 2005)

[0026] To assess the functional effects of aptamers on U87MG cells, 300.000 cells per 3.5-cm plate were treated with the indicated amount of RNA aptamers or the starting RNA G0 pool after a short denaturation-renaturation step. Cell extracts and immunoblotting analysis were performed as described (Cerchia L et al., 2003). The primary antibodies used were: anti-ERK1 (C-16) (Santa Cruz Biotechnology, Santa Cruz, California, United States) and anti-phospho-44/42 MAP kinase (indicated as anti-pERK) monoclonal antibodies (E10), anti-Akt, anti- phospho-Akt (Ser473, indicated as anti-pAkt), anti-PDK1, anti-phospho-PDK1 (Ser241, indicated as anti-pPDK1), anti-phospho-cyclin D1 (Thr286, indicated as p-cyclin D1), anti-cyclin D1, all from Cell Signaling, Beverly, Massachusetts, United States), anti-α-tubulin (DM 1A) (Sigma, St. Louis, MO). Four independent experiments were performed. Intensity of bands have been calculated using the NIH Image Program on at least two different expositions to assure the linearity of each acquisition. Fold values are expressed relative to the reference points, arbitrarily set to 1 (labelled with asterisk).

Whole-cell SELEX

[0027] Transcription was performed in the presence of 1 mM 2'F-Py, 1mM ATP, 1mM GTP, 10 mM DTT, 0.5 u/μl RNAse inhibitors (Amersham Pharmacia), 10 μCi/μl ^{32}P-αUTP (3000 Ci/mmol), 1 pmol/μl DNA and a mutant form of T7 RNA polymerase (2.5 u/μl T7 R&DNA polymerase, Epicentre) was used to improve yields. 2'F-Py RNAs were used because of their increased resistance to degradation by seric nucleases.

[0028] 2'F-Py RNAs (800-300 pmol) were heated at 85°C for 5 min in 1.5ml of DMEM serum free, snap-cooled on ice for 2 min, and allowed to warm up to 37°C. Before incubation with the cells, 13.5 ml of medium were added to RNA to reach a final volume of 15 ml.

Glioma as target

Counterselection against T98G cells

[0029] To avoid selecting for aptamers non-specifically recognizing the U87MG cell surface, the pool was first incubated for 30 min (up to round 9) or for 15 min (for the following rounds) at 37°C with 10^7 T98G cells (150-mm cell plate), and unbound sequences were recovered for the selection phase. This step was meant to select sequences recognizing specifically the U87MG cells.

Selection against U87MG cells

[0030] The recovered sequences were incubated with 10^7 U87MG cells for 30 min at 37°C and the U87MG-bound sequences were recovered after several washings with 5 ml of DMEM serum free by total RNA extraction (Ambion).

[0031] During the selection process, the authors progressively increased the selective pressure by increasing the number of washings (from one for the first cycle up to five for the last cycles) and by decreasing the incubation time (from 30 to 15 min from round 9). To follow the evolution of the pool the authors monitored the appearance of four-base restriction sites in the population by RFLP as previously described (Cerchia et al., 2005). After 14 rounds of selection, sequences were cloned with TOPO-TA cloning kit (Invitrogen, Carlsbad, California, United States) and analyzed.

NSCLC as target

Counter-selection on H460

[0032] To avoid selecting for aptamers non-specifically recognizing the Calu1 cell surface, the pool has been first incubated for 30 min (up to round 5) or for 15 min (for the following rounds) at 37°C with $2x10^6$ H460 cells (150-mm cell plate), and unbound sequences have been recovered for the selection phase.

Selection on Calu1

[0033] The sequences recovered from the counter-selection have been incubated with $2x10^6$ Calu1 cells for 30 min (up to round 5) or for 15 min (for the following rounds) at 37°C and the Calu1-bound sequences were recovered after several washings with DMEM serum free by total RNA extraction.

[0034] During the selection process, the authors progressively increased the selective pressure by: a) increasing the number of washings (from three for the first 9 cycles up to five for the last cycles); b) decreasing the incubation time (from 30 to 15 min starting from round 5); c) adding a second counter-selection step on H460 cells (from 1 to 2 counter-selections starting from round 4); d) adding polyI (polyinosinic acid) as a competitor for the last two selection cycles (round 13 and round 14).

Binding analysis

[0035] Binding of individual aptamers (or of the starting pool as a control) to U87MGcells and T98G cells was performed in 24-well plates in triplicate with 5'-^{32}P-labeled RNA. $3.5X10^4$ cells per well were incubated with various concentrations of individual aptamers in 200 μl of DMEM serum free for 20 min at RT in the presence of 100 μg/ml polyinosine as a nonspecific competitor (Sigma, St. Louis, MO). After five washings of 500 μl DMEM, bound sequences were recovered in 300 μl of SDS 1%, and the amount of radioactivity recovered was counted. The background values obtained with the starting pool were subtracted from the values obtained with the specific aptamers. Apparent Kd values for each aptamers were determined by Linewaver Burk analysis according to the equation:

$$1/[\text{complex}] = Kd/[Cmax] \times 1/[\text{aptamer}] + 1/[Cmax].$$

Sequences

[0036] GGGAGACAAGAAUAAACGCUCAA fixed primer (SEQ ID No. 1)

[0037] UUCGACAGGAGGCUCACAACAGGC fixed primer (SEQ ID No. 2)

[0038] Scheme 1 reports the aptamer's structure:

5′ GGGAGACAAGAAUAAACGCUCAA **(random sequence)** GUUCGACAGGAGGCUCACA ACAGGC **3′**

Scheme 1

Glioma as target

B20 (SEQ ID No. 3)
GGGAGACAAGAAUAAACGCUCAA**UCGUUUACAUUGUACUCUCCAUUAAUGACCCUCGGAUUG CUUAG**GUUCGACAGGAGGCUCACAACAGGC

D7 (SEQ ID No. 4)
GGGAGACAAGAAUAAACGCUCAA**ACUAUCAAUGCCUGACGCACGAUAAUCUUGCUGGUCUCA CAGAA**GUUCGACAGGAGGCUCACAACAGGC

C4 (SEQ ID No. 5)
GGGAGACAAGAAUAAACGCUCAA**CCGCAAUGACUACCGUCUUGCAGUUUUUAUAGCGUACUC UCAAUG**GUUCGACAGGAGGCUCACAACAGGC

C20 (SEQ ID No. 6)
GGGAGACAAGAAUAAACGCUCAA**CUGUCGAGCUUCAUUCAUGUGCUCACCGCUUACGCCUAA UGUCAU**GUUCGACAGGAGGCUCACAACAGGC

A2A21 (SEQ ID No. 7)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUCCCACGACAAUGUGAUACCUCU UAUGA**GUUCGACAGGAGGCUCACAACAGGC

C15 (SEQ ID No. 8)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUCCCACGACAAUGUGAUACCUCU UAUGA**GUUCGACAGGAGGCUCACAACAGGC

C24 (SEQ ID No. 9)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUCCCACGACAAUGUGAUACCUCU UAUAA**GUUCGACAGGAGGCUCACAACAGGC

C8 (SEQ ID No. 10)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUCCCACGACAAUGUGAUACCCCC UCAA**GUUCGACAGGAGGCUCACAACAGGC

D14 (SEQ ID No. 11)
GGGAGACAAGAAUAAACGCUCAA**CGAACGUUGUAUUUACUUGACCUCGCACUAGUUUAGCUU CCUACA**GUUCGACAGGAGGCUCACAACAGGC

D6 (SEQ ID No. 12)
GGGAGACAAGAAUAAACGCUCAA**CGAACGUUGUAUUUACCUGACCUCUCACUAGUUUAGCUU CCUACA**GUUCGACAGGAGGCUCACAACAGGC

B10 (SEQ ID No. 13)
GGGAGACAAGAAUAAACGCUCAA**UGCACAUGAGUAUUUAUUCAUCUCAAACGCUGACCUGCC AAUAA**GUUCGACAGGAGGCUCACAACAGGC

A7 (SEQ ID No. 14)
GGGAGACAAGAAUAAACGCUCAA**CCGUUGUUCUACAUGUCACUCAUCAUGCGAGUCUUUUGU**
CUACAGUUCGACAGGAGGCUCACAACAGGC

B2 (SEQ ID No. 15)
GGGAGACAAGAAUAAACGCUCAA**CCGUUGUUCUACAUGUCAGUCAUCAUGCGAGUCUUUUGU**
CUACAAGUUCGACAGGAGGCUCACAACAGGC

B19 (SEQ ID No. 16)
GGGAGACAAGAAUAAACGCUCAA**CCGUUGUUCUACAUGUCACUCAUCAUGCGAGUCUUUUUG**
UCUAGUUCGACAGGAGGCUCACAACAGGC

A6B15 (SEQ ID No. 17)
GGGAGACAAGAAUAAACGCUCAA**CCGUUGUUCUACAUGUCACUCAUCACGCGAGUCUUUUGU**
CUAAGUUCGACAGGAGGCUCACAACAGGC

C2 (SEQ ID No. 18)
GGGAGACAAGAAUAAACGCUCAA**UUGCCAAUACAGUUGAUCAUUGUCUUACCAUUGACUAGU**
ACCGUUCGACAGGAGGCUCACAACAGGC

C10 (SEQ ID No. 19)
GGGAGACAAGAAUAAACGCUCAA**CCCAAGUCAGUGAUUGGUAACUUUCACUUGACAAUAUCA**
AAUGCCGUUCGACAGGAGGCUCACAACAGGC

C1 (SEQ ID No. 20)
GGGAGACAAGAAUAAACGCUCAA**GCCUCUCAACGAUUAAUGUUUCAUUAACAUGAUCAAUCG**
CCUCAAGUUCGACAGGAGGCUCACAACAGGC

B22 (SEQ ID No. 21)
GGGAGACAAGAAUAAACGCUCAA**GCCUCUCAACGAUUAAUGUUUCGUUAACAUGAUCAAUCG**
CCUCAAGUUCGACAGGAGGCUCACAACAGGC

C5 (SEQ ID No. 22)
GGGAGACAAGAAUAAACGCUCAA**GGCAUUUGAUAUUGUCAAGUGAAAGUUACCAAUCACUGA**
CGUUCGACAGGAGGCUCACAACAGGC

D23 (SEQ ID No. 23)
GGGAGACAAGAAUAAACGCUCAA**UUAUUAACGUUAUCAUUGUUCUUCACUACUUGUAGUACC**
UUCGAGUUCGACAGGAGGCUCACAACAGGC

C22 (SEQ ID No. 24)
GGGAGACAAGAAUAAACGCUCAA**CGUUAUUACUAUGUAUCACAACGUGAACCCAUGUUGAAU**
CACAAGUUCGACAGGAGGCUCACAACAGGC

D2 (SEQ ID No. 25)
GGGAGACAAGAAUAAACGCUCAA**CCGUCUAUCGCGAAGCGUCUACUAUCCUUGUUCAAUUGU**
GACUUCGUUCGACAGGAGGCUCACAACAGGC

B13 (SEQ ID No. 26)
GGGAGACAAGAAUAAACGCUCAA**CUGCACAGCGUCCACACAACUUGAUCCACAAUUUUGAUG**
CCUUAUGUUCGACAGGAGGCUCACAACAGGC

B3 (SEQ ID No. 27)
GGGAGACAAGAAUAAACGCUCAA**CAACGAUGCUUGUUACGCGUAAUCUUAGUCACAUUGCUU**
GCGUGUUCGACAGGAGGCUCACAACAGGC

C9 (SEQ ID No. 28)
GGGAGACAAGAAUAAACGCUCAA**CAACGAUGCUUGUUAUGCGUAAUCUUAGUCACAUUGCUU**
GCGUGUUCGACAGGAGGCUCACAACAGGC

A20 (SEQ ID No. 29)
GGGAGACAAGAAUAAACGCUCAA**CACACGAUUGUUAUAAGCGCAUUACUCUCUGUCCCACUG**
UACUUGAGUUCGACAGGAGGCUCACAACAGGC

A2 (SEQ ID No. 30)
GGGAGACAAGAAUAAACGCUCAA**UAACGUGCUAUUCAGAACUUUGUCUGCCCACUUUUAGUG**
AACUCCAGUUCGACAGGAGGCUCACAACAGGC

D3 (SEQ ID No. 31)
GGGAGACAAGAAUAAACGCUCAA**UCCAUUUUGGAUGAUCGUUGUGAUUCUCGUAAUACAAGC**
CUUCAGUUCGACAGGAGGCUCACAACAGGC

C16 (SEQ ID No. 32)
GGGAGACAAGAAUAAACGCUCAA**CUAUCAAUAGUUGACAUCGUUCGCUGUCUAUCGCAAUAC**
UAUCCGUUCGACAGGAGGCUCACAACAGGC

C7 (SEQ ID No. 33)
GGGAGACAAGAAUAAACGCUCAA**CUUCAUGUUGAUCGCUUAUAAACUCACAUAGUUAGUCUC**
AUAAGUUCGACAGGAGGCUCACAACAGGC

C12 (SEQ ID No. 34)
GGGAGACAAGAAUAAACGCUCAA**UGAGUGUUAUCGAGUUGAUCGACAAUACAAUCUCACAAU**
ACCUUCGUUCGACAGGAGGCUCACAACAGGC

D9 (SEQ ID No. 35)
GGGAGACAAGAAUAAACGCUCAA**UACCAAACGCGCGGUUUUCGUCUCGUAAUAACCAAAUGC**
CUCUGAGUUCGACAGGAGGCUCACAACAGGC

A9 (SEQ ID No. 36)
GGGAGACAAGAAUAAACGCUCAA**UACCAAACGCGCAAUUUUCAUCUUGUAAUAACCAAAUGC**
CUCUGAGUUCGACAGGAGGCUCACAACAGGC

D21 (SEQ ID No. 37)
GGGAGACAAGAAUAAACGCUCAA**CAGUCGCGAAUUUUUUAUUCUUUCUUACAACAAAGCAUA**
GCCUCAGUUCGACAGGAGGCUCACAACAGGC

C18 (SEQ ID No. 38)
GGGAGACAAGAAUAAACGCUCAA**GAUUGCGGAUUCUCAUCUUUCCAACAACGAACUAGCCUC**
UACUAGUUCGACAGGAGGCUCACAACAGGC

C23 (SEQ ID No. 39)
GGGAGACAAGAAUAAACGCUCAA**UUGUCAACGAUCGAGCACGUUCUCACACAAAGCCUCUUA**
CUAUAU GUUCGACAGGAGGCUCACAACAGGC

C6 (SEQ ID No. 40)
GGGAGACAAGAAUAAACGCUCAA**CAAUCGCGUACGUUCUUGCGUAACAAACAGCCACUGUCA**
UAAAC GUUCGACAGGAGGCUCACAACAGGC

D13 (SEQ ID No. 41)
GGGAGACAAGAAUAAACGCUCAA**CGUUUACGCGUAAUCUUGUAAUUCACAUUCUCUCAACAA**
GCCUA GUUCGACAGGAGGCUCACAACAGGC

A4 (SEQ ID No. 42)
GGGAGACAAGAAUAAACGCUCAA**GACAUCAACAUCUCAACGAUCUUGUUACUCUCAACUCAA**
AUAGC GUUCGACAGGAGGCUCACAACAGGC

A5 (SEQ ID No. 43)
GGGAGACAAGAAUAAACGCUCAA**ACGUUACUCUUGCAACACAAACUUUAAUAGCCUCUUAUA**
GUUC GUUCGACAGGAGGCUCACAACAGGC

A10C13 (SEQ ID No. 44)
GGGAGACAAGAAUAAACGCUCAA**ACGUUACUCUUGCAACACCCAAACUUUAAUAGCCUCUUA**
UAGUUC GUUCGACAGGAGGCUCACAACAGGC

D18 (SEQ ID No. 45)
GGGAGACAAGAAUAAACGCUCAA**ACGUUACUCUUGCAACACCCAAACUUUAAUAGCCUCUUA**
CAGAA GUUCGACAGGAGGCUCACAACAGGC

D5 (SEQ ID No. 46)
GGGAGACAAGAAUAAACGCUCAA**UACAGCGCUAUUCUUCCAACCAAUCAUACCACCUUGUCA**
UGUUAA GUUCGACAGGAGGCUCACAACAGGC

C14 (SEQ ID No. 47)
GGGAGACAAGAAUAAACGCUCAA**CGAAUCGAAGCGAUAUUCCUUACCAAUUAAUUGUAUAGC**
CUUA GUUCGACAGGAGGCUCACAACAGGC

D19 (SEQ ID No. 48)
GGGAGACAAGAAUAAACGCUCAA**UGUUGCAACAUCGAGUCAGCGUGUUCUUCCAAGCCUCUA**
UAGAAC GUUCGACAGGAGGCUCACAACAGGC

D4 (SEQ ID No. 49)
GGGAGACAAGAAUAAACGCUCAA**CAUCGAAUACAGCCUUUAAUCCAACCUCCAAUUUCAAUC**
GACUAA GUUCGACAGGAGGCUCACAACAGGC

B7 (SEQ ID No. 50)
GGGAGACAAGAAUAAACGCUCAA**UUCAGCGAUGUUCUAAUCACCACAUAACAAACUAUAGCC**
AGACCU GUUCGACAGGAGGCUCACAACAGGC

B8 (SEQ ID No. 51)
GGGAGACAAGAAUAAACGCUCAA**UGAUCGUUGAAUUCAACUGUCCACUUAACAAAUUUCAGC**
CACUAA GUUCGACAGGAGGCUCACAACAGGC

D22 (SEQ ID No. 52)
GGGAGACAAGAAUAAACGCUCAA**UUCGUGUCAACUCAACCAACCAAGCCUUCUGACGUACAC**
UAAG GUUCGACAGGAGGCUCACAACAGGC

C3C11D10 (SEQ ID No. 53)
GGGAGACAAGAAUAAACGCUCAA**ACAGCGAUUCGAUCUCUACCCACAACACAAAUGCCUUCA**
CACAUA GUUCGACAGGAGGCUCACAACAGGC

B17 (SEQ ID No. 54)
GGGAGACAAGAAUAAACGCUCAA**UGCGCGAAUUCUAUCCGUAUGCAAUUCAUGCAUACAUUC**
CAACUA GUUCGACAGGAGGCUCACAACAGGC

B14 (SEQ ID No. 55)
GGGAGACAAGAAUAAACGCUCAA**UUAGAAUUCUAAUUUGAUAAUAUUACUUGCCGCCUCCAC**
GAACAC GUUCGACAGGAGGCUCACAACAGGC

A3A1B4B8C19D11 (SEQ ID No. 56)
GGGAGACAAGAAUAAACGCUCAA**UGAUUUUGCAGCACUUCUUGUUAUCUUAACGAACUGUUG**
AUGAGUUCGACAGGAGGCUCACAACAGGC

B16 (SEQ ID No. 57)
GGGAGACAAGAAUAAACGCUCAA**CUAAGAGGUUGACGCUUAGCACUUCCAGUAACCUAAGCC**
UUCUA GUUCGACAGGAGGCUCACAACAGGC

B4 (SEQ ID No. 58)
GGGAGACAAGAAUAAACGCUCAA**UGUUUGACUUGAUUCUCUAGCUUACAAAUGUUAACAUCU**
GCAAA GUUCGACAGGAGGCUCACAACAGGC

D12 (SEQ ID No. 59)
GGGAGACAAGAAUAAACGCUCAA**UGUCUUGUUUAUUCGAACUCACAUUAACAACAAUGAUUA**
GACGGC GUUCGACAGGAGGCUCACAACAGGC

C21 (SEQ ID No. 60)
GGGAGACAAGAAUAAACGCUCAA**CCGCAACAAGAUUGACGGCUUGCGUAAAUUCACAAGAUU**
UCAUU GUUCGACAGGAGGCUCACAACAGGC

D15 (SEQ ID No. 61)
GGGAGACAAGAAUAAACGCUCAA**CUGUGACGACAGUUAAGAUCGUAUUCUGCCACCAUACCU**
GUUGUA GUUCGACAGGAGGCUCACAACAGGC

D1D20 (SEQ ID No. 62)

GGGAGACAAGAAUAAACGCUCAA**UUCACACACUCAAUUGAACGGUGAUUCAAGUUAUUAGCA**
GCCUCA GUUCGACAGGAGGCUCACAACAGGC

NSCLC as target

DL1 (SEQ ID No. 63)
GGGAGACAAGAAUAAACGCUCAA**ACGCUUGUCUUGUUUUCGUGAGCUAAAGUAUCAGUCAGA**
GGCAAUUCGACAGGAGGCUCACAACAGGC

BL8 (SEQ ID No. 64)
GGGAGACAAGAAUAAACGCUCAA**CCGUUGUUCUACAUGUCACUCAUCACGCGAGUCUUUUGU**
CUACAUUCGACAGGAGGCUCACAACAGGC

DL2 (SEQ ID No. 65)
GGGAGACAAGAAUAAACGCUCAA**CCGUUGUUCUACAUGUCACUCAUCAUACGAGUCUUUUGU**
CUAUUCGACAGGAGGCUCACAACAGGC

AL1-CL6-CL8-EL4 (SEQ ID No. 66)
GGGAGACAAGAAUAAACGCUCAA**CCGUUGUUCUACAUGUCACUCAUCAUGCGAGUCUUUUGU**
CUAAUUCGACAGGAGGCUCACAACAGGC

HL1 (SEQ ID No. 67)
GGGAGACAAGAAUAAACGCUCAA**CGAGACUUUAACGUUUGACUUGUUUGACCAAAUGUGUGA**
UACCUUCGACAGGAGGCUCACAACAGGC

GL2B (SEQ ID No. 68)
GGGAGACAAGAAUAAACGCUCAA**GUCAAAUGGGCGUAUUACGUAAAUUUUCCGGCAGUAUGU**
GAAGCAUUCGACAGGAGGCUCACAACAGGC

AL8 (SEQ ID No. 69)
GGGAGACAAGAAUAAACGCUCAA**UGAUUUUGCAGCACUUCUCGUUAUCUUAGCGAGCUGUUG**
AUGAUUCGACAGGAGGCUCACAACAGGC

BL2 (SEQ ID No. 70)
GGGAGACAAGAAUAAACGCUCAA**UGAUUUUGCAGCACUUCUUGUUAUCUUAACGAGCUGUUG**
AUGGUUCGACAGGAGGCUCACAACAGGC

DL8-EL1-FL8 (SEQ ID No. 71)
GGGAGACAAGAAUAAACGCUCAA**CGUGCAACGCACAAAUUCUUGAUCAUCUCAAUGAUGUGU**
GCUUUCGACAGGAGGCUCACAACAGGC

EL2 (SEQ ID No. 72)
GGGAGACAAGAAUAAACGCUCAA**CGUGCAACGCACAAAUUCUUGAUCAUCUCAAUGAUGUGU**
GUCUUUCGACAGGAGGCUCACAACAGGC

DL6 (SEQ ID No. 73)
GGGAGACAAGAAUAAACGCUCAA**CGUGCGACAUACAAAUUCUUGAUCAUCCCAAUGAUGUGU**
GCUUUCGACAGGAGGCUCACAACAGGC

EL3-GL4 (SEQ ID No. 74)
GGGAGACAAGAAUAAACGCUCAA**CGUGCGACAUACAAAUUCUUGAUCAUCUCAAUGAUGUGU**
GCUUUCGACAGGAGGCUCACAACAGGC

CL5-GL2A (SEQ ID No. 75)
GGGAGACAAGAAUAAACGCUCAA**UACCAAACGCGCAAUUUUCAUCUUGUAAUAACCAAAUGC**
CUCUGAUUCGACAGGAGGCUCACAACAGGC

AL5 (SEQ ID No. 76)
GGGAGACAAGAAUAAACGCUCAA**UACCAAACGCGCGAUUUUCAUCUUGUAAUAACCAAAUGC**
CUCUGAUUCGACAGGAGGCUCACAACAGGC

BL5 (SEQ ID No. 77)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUCCCACAACAAUGUGAUACCUCU**
UAUGAUUCGACAGGAGGCUCACAACAGGC

AL6-BL9-CL9-DL7 (SEQ ID No. 78)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUCCCACGACAAUGUGAUACCUCU**
UAUGAUUCGACAGGAGGCUCACAACAGGC

CL7 (SEQ ID No. 79)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUCCCACGACAAUGUGAUACCUCU**
UAUGGUUCGACAGGAGGCUCACAACAGGC

GL9 (SEQ ID No. 80)
GGGAGACAAGAAUAAACGCUCAA**UUGCAUUUACUCGAUGUUCCACAACAAUGUGAUACCUCU**
UAUGAUUCGACAGGAGGCUCACAACAGGC

EL7 (SEQ ID No. 81)
GGGAGACAAGAAUAAACGCUCAA**AACUCUGGGGCGCUAUUCUCAUCGCAAACCCAACCGUUG**
UGUACCUUUCGACAGGAGGCUCACAACAGGC

FL1 (SEQ ID No. 82)
GGGAGACAAGAAUAAACGCUCAA**ACGUGCGACAUACAAAUUCUUGAUCAUCUCAAUGAUGUG**
UGCUUUCGACAGGAGGCUCACAACAGGC

BL3 (SEQ ID No. 83)
GGGAGACAAGAAUAAACGCUCAA**GUCGUAAGGUUGCGUAUGUGUUCGUGUAAUCUCAUUGCG**
AGCUCUUCGACAGGAGGCUCACAACAGGC

AL4 (SEQ ID No. 84)
GGGAGACAAGAAUAAACGCUCAA**GUCGUAAGGUUGUGUAUGUGUUCGUGUAAUCUCAUUGCG**
AGCUCUUCGACAGGAGGCUCACAACAGGC

EL6 (SEQ ID No. 85)
GGGAGACAAGAAUAAACGCUCAA**GUUGUGCCAUGUUAGCGCACAAUUUGUAAUUCAAGAGCG**
CAAGUUCGACAGGAGGCUCACAACAGGC

FL5 (SEQ ID No. 86)
GGGAGACAAGAAUAAACGCUCAA**UGCCUACUCUUGUCAUCUCUAGAGCCAAAUACAAGCGUU**

AACAUUCGACAGGAGGCUCACAACAGGC

FL4 (SEQ ID No. 87)
GGGAGACAAGAAUAAACGCUCAA**UGGUUGAAGCAUGAGUCGUUCUUCUUGCCAUGUGAAAGC**
UUUCGACAGGAGGCUCACAACAGGC

FL2 (SEQ ID No. 88)
GGGAGACAAGAAUAAACGCUCAA**UGGUUGCAAAAUACAUGAACGUCAAUUUUCAGUCUUGAU**
ACCUGUUCGACAGGAGGCUCACAACAGGC

EL8 (SEQ ID No. 89)
GGGAGACAAGAAUAAACGCUCAA**AUGCCUACUCUUGUCAUCUCUGAGCCAAAUACAAGCGUU**
AACAUUCGACAGGAGGCUCACAACAGGC

GL1 (SEQ ID No. 90)
GGGAGACAAGAAUAAACGCUCAA**CGAUUUGUGGCGACAGGUUAAACGUCGCUUCAAUUUCGC**
AGCAUUCGACAGGAGGCUCACAACAGGC

DL5 (SEQ ID No. 91)
GGGAGACAAGAAUAAACGCUCAA**CGGUACAUGCGUUGAUUUUCUUGCACACAGCCUCUAUAA**
CAACUUUCGACAGGAGGCUCACAACAGGC

FL3 (SEQ ID No. 92)
GGGAGACAAGAAUAAACGCUCAA**AUGAAUCGGAAAGCGCAAUCUUGAGUUCUCCUACCUUUU**
GUGAUUCGACAGGAGGCUCACAACAGGC

DL9 (SEQ ID No. 93)
GGGAGACAAGAAUAAACGCUCAA**CGACUUGUAUGUCUUGAUGUGAAUCUUCUAAUCUACCAU**
GAGCAUUCGACAGGAGGCUCACAACAGGC

FL7 (SEQ ID No. 94)
GGGAGACAAGAAUAAACGCUCAA**GCCUCUCAACGAUUAAUGUUUCAUUAACAUGAUCAAUCG**
CCUCAAUUCGACAGGAGGCUCACAACAGGC

AL9 (SEQ ID No. 95)
GGGAGACAAGAAUAAACGCUCAA**GGUCAAAAACGUUUGCUUGUUUUCAGGAUACAAUGUGGA**
GCCAUAUUCGACAGGAGGCUCACAACAGGC

FL9 (SEQ ID No. 96)
GGGAGACAAGAAUAAACGCUCAA**UUCAGCGCAACUGUUCGUCUUUCCACGGCUGUGAGACUU**
CAGAAUUCGACAGGAGGCUCACAACAGGC

EL9 (SEQ ID No. 97)
GGGAGACAAGAAUAAACGCUCAA**UUCAGCGCAACUGUUCGUCUUUCCACGGCUGUGAGACUU**
CAGGAUUCGACAGGAGGCUCACAACAGGC

DL3-GL7 (SEQ ID No. 98)
GGGAGACAAGAAUAAACGCUCAA**UUCAGCGCAACUGUUCGUCUUUCCACGGCUGUGAGACUU**
CGGAAUUCGACAGGAGGCUCACAACAGGC

CL1-GL5 (SEQ ID No. 99)
GGGAGACAAGAAUAAACGCUCAA**UUCAGCGCAACUGUUCGUCUUUCCAUGGCUGUGAGACUU**
CAGAAUUCGACAGGAGGCUCACAACAGGC

DL4 (SEQ ID No. 100)
GGGAGACAAGAAUAAACGCUCAA**UUUGUUGCGAAUCGCACAUAUUGGACGUUCUGUUUGUGU**
GAGUAUUCGACAGGAGGCUCACAACAGGC

BL6 (SEQ ID No. 101)
GGGAGACAAGAAUAAACGCUCAA**UUUGUUGCGAAUCGCACGUAUUGGACGUUCUGUUUGUGU**
GAGUAUUCGACAGGAGGCUCACAACAGGC

GL8 (SEQ ID No. 102)
GGGAGACAAGAAUAAACGCUCAA**UUUGUUGCGAAUUGCACAUAUUGGACGUUCUGUUGUGUG**
AGUAUUCGACAGGAGGCUCACAACAGGC

CL3 (SEQ ID No. 103)
GGGAGACAAGAAUAAACGCUCAA**GAACGUUGUAUUUACUUGACCUCUCGCUAGUUUAGCUUU**
CUACAUUCGACAGGAGGCUCACAACAGGC

BL7 (SEQ ID No. 104)
GGGAGACAAGAAUAAACGCUCAA**UCCAUUUUGGAUGAUUGUUGUGAUUCUCGUAAUACAAGC**
CUUCAUUCGACAGGAGGCUCACAACAGGC

CL4 (SEQ ID No. 105)
GGGAGACAAGAAUAAACGCUCAA**CGACACGUUGCCAGCCGGAGCCUUAGUAACGUGCUUUGA**
UGUCGAUUCGACAGGAGGCUCACAACAGGC

EXAMPLES

EXAMPLE 1: Whole cell SELEX using Glioma cells: Differential whole cell SELEX

Enrichment of selection for a complex target, RFLP, enrichment of recovery, differential binding on different cell lines

[0039] In order to isolate cell specific ligands for a given tumor cell phenotype, the authors used as a model system, stable human glioma cell lines. Stable cell lines have the advantage that they can be kept under well controlled growth conditions and that they remain stable all along the SELEX procedure. The authors used as target for the selection steps the human malignant glioma cell line, U87MG and for the counterselection steps the T98G. These two cell lines differ for the potential to form tumors in nude mice and for resistance to radiation-induced cell death. U87MG being highly tumorigenic and radio-resistant while the T98G are poorly tumorigenic and sensitive to radiations. On the other hand, these cell lines share the same altered cellular pathways as both harbor $p14^{arf}$/p16 deletion and PTEN mutation. The major difference found between the two cell lines is the levels of ErbB2 and pErk , that are higher in U87MG than in T98G, while pAkt and NCAM levels are similar (data not shown). The relative levels of these four molecules were monitored at each cycle of the SELEX procedure to verify and standardize the growth conditions of the cells.

[0040] A library of 2'Fluoro Pyrimidines (2'F-Py), nuclease-resistant RNAs was utilized for differential SELEX against intact cells (Fig. 1). Each selection step on U87MG cells, was preceded by one or two counterselection steps against the T98G cells.

[0041] The method of the present invention is particularly efficient in selecting highly selective aptamers since at each SELEX cycle, the pool of aptamers is deprived of aptamers that recognize common cellular antigens present at high levels on the surface of both control and target cell lines. As a consequence, in the pool is impoverished of unwanted sequences, thus the aptamer for the specific rare antigens will be able to bind its target even if embedded in a complex target. The protocol consists of applying at each round one or more counterselection steps before each positive selection

step.

[0042] During the selection process, the authors progressively increased the selective pressure by changing both incubation and washing conditions (Fig. 2A). Following each round the authors monitored the evolution of the pool by Restriction Fragment Length Polymorphism analysis (RFLP). After 8 rounds of selection, some sequences were predominantly amplified and dominated in abundance the aptamer pool, resulting in discrete restriction bands. During rounds 13 and 14, RFLP profiles remain unchanged, indicating an evolution of the sequence distribution in the library (Fig 2B).

Cloning and distribution of individual sequences

[0043] After 14 rounds of selection, the pool, named G14, was enriched for aptamers that preferentially bind to U87MG cells when compared with the in vitro binding efficiency on T98G cells and compared to the binding of the naive starting pool (Fig 3).

[0044] A panel of 71 sequences were cloned from the pool G14 and aptamers were grouped in families based on their primary sequence similarity (Fig 5 and Fig 6). The authors identified The authors identified ten families of highly related aptamers that together cover more than 46% (34 aptamers) of the all individual sequences obtained from the selection; an individual sequence, C19 (also codified as, A3, A1, B4, B8, D11), dominated the selection and constituted 8% of all the clones; five other sequences, C3 (also codified as C11 and D10), A6 (also codified as B15), A10 (also codified as C13) D1 (also codified as D20), A2 (also codified as A21) represented together more than 15% of the clones. The remaining 37 sequences were poorly related each other.

[0045] Using the starting pool as a control, binding of individual aptamers to U87MG and T98G cells was then performed. In order to screen for individual ligand aptamers that efficiently target U87MG cells, at least one member for each family (a total of 21 aptamers were tested) was first analysed at 500nM. At this concentrations, 8 aptamers display up to a five-fold increase of binding to U87MG cells with respect to the starting pool, the remaining 13 aptamers having no specific binding for U87MG. The results are shown in Fig 9.

[0046] As shown in Table 1, these 8 sequences bind at high affinity (with Kd ranging between 38 nM and 710 nM) the U87MG cells and have no or low affinity for T98G (not shown).

Table 1. Kd (nM) and *Cmax* (pM) of the best sequences

Aptamer	Kd (nM)	C*max* (pM)
B15	102 ± 12	3400 ± 408
B22	221 ± 25	4310 ± 495
D20	710 ± 40	20000 ± 3400
A5	44 ± 4	290 ± 26
D9	43.7 ± 7	2100 ± 330
C13	38 ± 3	2900 ± 232
C19	63 ± 9	290 ± 41
A9	190 ± 20	3410 ± 340

Bioinformatic analysis of individual sequences

[0047] Four of the eight aptamers considered (B22, B15, C19 and D20) have unrelated primary sequences and predicted 2D folded structures. Two of them (C13 and A5) differ for the presence of two cytosine residues [C42 C43] that are only present in C13 whose presence however doesn't alter the affinity for the target cells (see Table 1). Consistently, the predicted secondary structures are unaltered by the presence of C42, C43 (Fig 10). The opposite situation was found in another couple of aptamers (A9 and D9) that poorly differ in their primary structure but display different predicted secondary structures (Fig 10). Interestingly, such difference changes also the binding affinity by 4,5 times. In fact, binding affinities reported in Table 1 shows a Kd of 43.7 nM for D9 and a Kd of 190 nM for A9. In Fig 11 were reported the relative binding values at the same concentration for all aptamers, i.e. 50 nM.

Binding on unrelated and glioma cell lines

[0048] The identification of a small set of aptamers that may distinguish the U87MG cells from the T98G cells raises the obvious question of whether these aptamers may also bind to other cell types. To this aim, the authors determined the relative binding potential of each aptamer to several cell lines. The authors first determined the cell type specificity by measuring the binding of each aptamer on a panel of unrelated cell lines. They found that the aptamers did not bind

to fibroblast NIH3T3 and did not recognize other cancer types including human neuroblastoma (SKNBE and SHSY5Y), lung (H460 and Calu1) and breast (MCF7 and SKBR3) cells (Fig. 11B). Further, the aptamers bind to different extents to glioma cell lines (U87MG, T98G, U251MG, TB10, LN-18, LN-229 and U87MG.ΔEGFR) characterised by different malignant phenotypes (Fig 11A). At that aptamers concentration each glioma cell line has a distinct pattern of binding (see Legend). At these experimental conditions, all aptamers have good binding with the highly tumorigenic cell lines (U87MG, LN-229, U87MG.ΔEGFR and TB10), the aptamers C19 binds to all cell lines except the non tumorigenic T98G, and B15 binds only the four highly tumorigenic cell lines. Thus the pattern of binding of five of these aptamers (for example, C13, B15, C19, A9 and D9) is sufficient to distinguish two cell lines (see Fig 11A).

Biological activities

[0049] Biological activities of each aptamer have been thus verified in U87MG cells. As previously demonstrated for the anti RET receptor tyrosine kinase D4 aptamer, high affinity aptamer binding to an extracellular receptor may inhibit activity of downstream transducing molecules, as ERK family members. Therefore, the authors first determined whether any of the U87MG specific aptamer may interfere with the presence of the phosphorylated active Akt and Erk 1/2. Surprisingly, five of the tested aptamers (A9, D9, C13, A5 and B22) inhibited ERK phosphorylation, compared to the control starting pool and to the other aptamers (B15, C19, D20) (Fig 12A). On the other hand no aptamer had any relevant effect on the phosphorylation of Akt and PDK1, likely because the U87MG harbor a mutated inactive PTEN, a phosphatase that negatively regulates the levels of Akt phosphorylation (Fig 11B).

[0050] To further confirm the biological activity of A9, D9, C13, A5 and B22, the authors determined the extent of inhibition of expression of the cell cycle-related protein, cyclin D1 and of phosphorylation of ERK 1/2 upon treatment of U87MG cells with aptamers for increasing time periods. As shown in Fig. 13A, treatment with cognate aptamers either A9 and D9, or C13 and A5, inhibits at similar extents basal cyclin D1 expression and phosphorylation in a time dependent manner. Further, treating cells with the aptamer B22 resulted as well in a stronger and more rapid inhibition of cyclin D1 reaching around 26% at 1 h.

[0051] As shown in Fig. 13B treatment with the same five aptamers caused a similar time dependent inhibition of Erk phosphorylation. Inhibition being more rapid with D9 than A9, thus according to their respective Kd values (see Table 1), and, as expected, at comparable extents treating with the highly related C13 and A5 aptamers.

EXAMPLE 2: Whole-cell SELEX to isolate RNA-aptamers against TRAIL-resistant NSCLC

[0052] To extend the validity of the whole-cell SELEX approach to a different cell system, the authors have also performed the selection on NSCLC cells.

[0053] In order to generate RNA-aptamers able to discriminate between TRAIL-resistant and TRAIL-sensitive cell phenotype, the authors have selected for the SELEX method, two different cell lines of human lung carcinoma among four different NSCLC: A459, Calu1, H460, and A549.

[0054] These NSCLC have been extensively characterized for their resistance to the cytotoxic effects of TRAIL and it has been established that the human lung Calu1 (*epidermoid lung carcinoma*) cells (p53 null) are resistant to TRAIL, while the H460 (*lung epithelial cell carcinoma*) cells (wild type p53) are highly sensitive to TRAIL (Zanca C. et al., 2008).

[0055] Furthermore, these four NSCLC have been characterized for their expression of molecules participating in the apoptotic process and for their different sensitivity to the chemotherapies that are currently in use for the treatment of lung cancer: paclitaxel, cisplatinum, carboplatin, navelbine and gemcitabine. The experiments revealed that the cell lines tested are all resistant to cisplatinum, cambomplatinum, navelbine and gemcitabine. By contrast, they are characterised by different sensitivity to paclitaxel: two cell lines are resistant (A459, Calu1) and two are sensitive (H460, and A549). As a further characterization, the authors have performed immunoblotting analyses on cell extracts from the four cell lines and among them, Calu1 and H460 cells showed the highest and the lowest, respectively, levels of the analyzed proteins, for examples EGFR, PED, c-FLIP (not shown).

[0056] The authors applied the same approach as for glioma cells by using a selection step on Calu1 cells preceded by counter-selection on H460.

[0057] RFLP analysis performed on the pool from each round of selection (named L1 to L14) and on the starting pool (L0) revealed stabilized profiles following 14 rounds of selection.

EXAMPLE 3:Whole cell SELEX using NSCLC cells: Differential whole cell SELEX

Enrichment of selection for a complex target, RFLP, enrichment of recovery, differential binding on different cell lines

[0058] In order to isolate cell specific ligands for a given tumor cell phenotype, the authors used as a model system, stable human NSCLC cell lines. Stable cell lines have the advantage that they can be kept under well controlled growth

conditions and that they remain stable all along the SELEX procedure. The authors used as target for the selection steps the human malignant NSCLC cell line, Calu1 and for the counterselection steps the H460. These NSCLC have been extensively characterized for their resistance to the cytotoxic effects of TRAIL and it has been established that the human lung Calu1 (*epidermoid lung carcinoma*) cells (p53 null) are resistant to TRAIL, while the H460 (*lung epithelial cell carcinoma*) cells (wild type p53) are highly sensitive to TRAIL (Zanca C. et al., 2008).

[0059] Furthermore, these two NSCLC have been characterized for their expression of molecules participating in the apoptotic process and for their different sensitivity to the chemotherapies that are currently in use for the treatment of lung cancer: paclitaxel, cisplatinum, carboplatin, navelbine and gemcitabine. The experiments revealed that the cell lines tested are both resistant to cisplatinum, cambomplatinum, navelbine and gemcitabine. By contrast, they are characterised by different sensitivity to paclitaxel: one is resistant (Calu1) and one is sensitive (H460). As a further characterization, the authors have performed immunoblotting analyses on cell extracts from two cell lines and Calu1 and H460 cells showed the highest and the lowest, respectively, levels of the analyzed proteins, for examples EGFR, PED, c-FLIP (not shown)

[0060] A library of 2'Fluoro Pyrimidines (2'F-Py), nuclease-resistant RNAs was utilized for differential SELEX against intact cells (Fig. 1). Each selection step on Calu1 cells was preceded by one or two counterselection steps against the H460 cells.

[0061] The method of the present invention is particularly efficient in selecting highly selective aptamers since at each SELEX cycle, the pool of aptamers is deprived of aptamers that recognize common cellular antigens present at high levels on the surface of both control and target cell lines. As a consequence, in the pool is impoverished of unwanted sequences, thus the aptamer for the specific rare antigens will be able to bind its target even if embedded in a complex target. The protocol consists of applying at each round one or more counterselection steps before each positive selection step.

[0062] During the selection process, the authors progressively increased the selective pressure by changing both incubation and washing conditions (Fig. 2A). Following each round the authors monitored the evolution of the pool by Restriction Fragment Length Polymorphism analysis (RFLP). After 5 rounds of selection, some sequences were predominantly amplified and dominated in abundance the aptamer pool, resulting in discrete restriction bands. During rounds 12, 13 and 14, RFLP profiles remain unchanged, indicating an evolution of the sequence distribution in the library (Fig 2B).

Cloning and distribution of individual sequences

[0063] After 14 rounds of selection, the pool, named L14, was enriched for aptamers that preferentially bind to Calu1 cells when compared with the in vitro binding efficiency on H460 cells and compared to the binding of the naive starting pool (Figure 4).

[0064] A panel of 42 sequences were cloned from the pool L14 and aptamers were grouped in families based on their primary sequence similarity (Fig 7 and 8). The authors identified 18 families of aptamers: 5 families cover more than 60% of all the individual sequences obtained from the selection; an individual sequence, C19 (also codified as, A3, A1, B4, B8, D11) dominated the selection and constituted 9 % of all the clones; five sequences C19 (also codified as, A3, A1, B4, B8, D11), C3 (also codified as C11 and D10), A6 (also codified as B15), A10 (also codified as C13) D1 (also codified as D20) represented together more than 20% of the clones.

REFERENCES

[0065]

Tuerk C. Gold L. Science 1990, 249, 505-510
Ellington and Szostak Nature 1990, 346: 818-22
Green LS et al., Chem Biol. 1995, 2(10): 683-95
Tasset DM, Kubik MF, Steiner J., Mol. Biol. 1997, 272(5): 688-98
Ruckman J, et al., J. Biol. Chem. 1998, 273(32):20556-67
Cerchia et al., PLoS Biol. 2005, 3(4):e123
Zuker, M. Nucleic Acids Res. 2003, 31, 3406-3415
Zanca C. et al., J Cell Mol. Med. 2008, Feb 15
Esposito CL, et al., PLoS ONE 2008, 3(2):e1643
Buckley MF et al., Oncogene 1993, 8(8):2127-33
Cerchia L, et al., Biochem J. 2003, 372: 897-903
Ishii N et al., Brain Pathol. 1999, 9: 469-79
Nishikawa R et al., Proc Nati Acad Sci. 1994, 91: 7727-31
Pallini R et al (2006) Int. J. Cancer 118: 2158-67

SEQUENCE LISTING

<110> Consiglio Nazionale delle Ricerche
De Franciscis, Vittorio
Cerchia, Laura
Condorelli, Gerolama

<120> Method for obtaining oligonucleotide aptamers and uses thereof

<130> BE 102859

<160> 105

<170> PatentIn version 3.3

<210> 1
<211> 23
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 1
gggagacaag aauaaacgcu caa 23

<210> 2
<211> 24
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 2
uucgacagga ggcucacaac aggc 24

<210> 3
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 3
gggagacaag aauaaacgcu caaucguuua cauuguacuc uccauuaaug acccucggau 60
ugcuuagguu cgacaggagg cucacaacag gc 92

<210> 4
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 4
gggagacaag aauaaacgcu caaacuauca augccugacg cacgauaauc uugcuggucu 60
cacagaaguu cgacaggagg cucacaacag gc 92

<210> 5
<211> 93

<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 5
gggagacaag aauaaacgcu caaccgcaau gacuaccguc uugcaguuuu uauagcguac 60

ucucaauggu ucgacaggag gcucacaaca ggc 93

<210> 6
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 6
gggagacaag aauaaacgcu caacugucga gcuucauuca ugugcucacc gcuuacgccu 60

aaugucaugu ucgacaggag gcucacaaca ggc 93

<210> 7
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 7
gggagacaag aauaaacgcu caauugcauu uacucgaugu cccacgacaa ugugauaccu 60

cuuaugaguu cgacaggagg cucacaacag gc 92

<210> 8
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 8
gggagacaag aauaaacgcu caauugcauu uacucgaugu cccacgacaa ugugauaccu 60

cuuaugaguu cgacaggagg cucacaacag gc 92

<210> 9
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 9
gggagacaag aauaaacgcu caauugcauu uacucgaugu cccacgacaa ugugauaccu 60

cuuauaaguu cgacaggagg cucacaacag gc 92

<210> 10

<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 10
gggagacaag aauaaacgcu caauugcauu uacucgaugu cccacgacaa ugugauaccc 60

ccucaaguuc gacaggaggc ucacaacagg c 91

<210> 11
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 11
gggagacaag aauaaacgcu caacgaacgu uguauuuacu ugaccucgca cuaguuuagc 60

uuccuacagu ucgacaggag gcucacaaca ggc 93

<210> 12
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 12
gggagacaag aauaaacgcu caacgaacgu uguauuuacc ugaccucuca cuaguuuagc 60

uuccuacagu ucgacaggag gcucacaaca ggc 93

<210> 13
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 13
gggagacaag aauaaacgcu caaugcacau gaguauuuau ucaucucaaa cgcugaccug 60

ccaauaaguu cgacaggagg cucacaacag gc 92

<210> 14
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 14
gggagacaag aauaaacgcu caaccguugu ucuacauguc acucaucaug cgagucuuuu 60

gucuacaguu cgacaggagg cucacaacag gc 92

<210> 15
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 15
gggagacaag aauaaacgcu caaccguugu ucuacauguc agucaucaug cgagucuuuu 60

gucuacaagu ucgacaggag gcucacaaca ggc 93

<210> 16
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 16
gggagacaag aauaaacgcu caaccguugu ucuacauguc acucaucaug cgagucuuuu 60

ugucuaguuc gacaggaggc ucacaacagg c 91

<210> 17
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 17
gggagacaag aauaaacgcu caaccguugu ucuacauguc acucaucacg cgagucuuuu 60

gucuaaguuc gacaggaggc ucacaacagg c 91

<210> 18
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 18
gggagacaag aauaaacgcu caauugccaa uacaguugau cauugucuua ccauugacua 60

guaccguucg acaggaggcu cacaacaggc 90

<210> 19
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 19
gggagacaag aauaaacgcu caacccaagu cagugauugg uaacuuucac uugacaauau 60

caaaugccgu ucgacaggag gcucacaaca ggc 93

<210> 20
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 20
gggagacaag aauaaacgcu caagccucuc aacgauuaau guuucauuaa caugaucaau 60

cgccucaagu ucgacaggag gcucacaaca ggc 93

<210> 21
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 21
gggagacaag aauaaacgcu caagccucuc aacgauuaau guuucguuaa caugaucaau 60

cgccucaagu ucgacaggag gcucacaaca ggc 93

<210> 22
<211> 88
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 22
gggagacaag aauaaacgcu caaggcauuu gauauuguca agugaaaguu accaaucacu 60

gacguucgac aggaggcuca caacaggc 88

<210> 23
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 23
gggagacaag aauaaacgcu caauuauuaa cguuaucauu guucuucacu acuuguagua 60

ccuucgaguu cgacaggagg cucacaacag gc 92

<210> 24
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 24
gggagacaag aauaaacgcu caacguuauu acuauguauc acaacgugaa cccauguuga 60

aucacaaguu cgacaggagg cucacaacag gc 92

<210> 25
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 25
gggagacaag aauaaacgcu caaccgucua ucgcgaagcg ucuacuaucc uuguucaauu 60

gugacuucgu ucgacaggag gcucacaaca ggc 93

<210> 26
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 26
gggagacaag aauaaacgcu caacugcaca gcguccacac aacuugaucc acaauuuuga 60

ugccuuaugu ucgacaggag gcucacaaca ggc 93

<210> 27
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 27
gggagacaag aauaaacgcu caacaacgau gcuuguuacg cguaaucuua gucacauugc 60

uugcguguuc gacaggaggc ucacaacagg c 91

<210> 28
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 28
gggagacaag aauaaacgcu caacaacgau gcuuguuaug cguaaucuua gucacauugc 60

uugcguguuc gacaggaggc ucacaacagg c 91

<210> 29
<211> 94
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 29
gggagacaag aauaaacgcu caacacacga uuguuauaag cgcauuacuc ucugucccac 60

uguacuugag uucgacagga ggcucacaac aggc 94

<210> 30
<211> 94
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 30
gggagacaag aauaaacgcu caauaacgug cuauucagaa cuuugucugc ccacuuuuag 60

ugaacuccag uucgacagga ggcucacaac aggc 94

<210> 31
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 31
gggagacaag aauaaacgcu caauccauuu uggaugaucg uugugauucu cguaauacaa 60

gccuucaguu cgacaggagg cucacaacag gc 92

<210> 32
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 32
gggagacaag aauaaacgcu caacuaucaa uaguugacau cguucgcugu cuaucgcaau 60

acuauccguu cgacaggagg cucacaacag gc 92

<210> 33
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 33
gggagacaag aauaaacgcu caacuucaug uugaucgcuu auaaacucac auaguuaguc 60

ucauaaguuc gacaggaggc ucacaacagg c 91

<210> 34
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 34
gggagacaag aauaaacgcu caaugagugu uaucgaguug aucgacaaua caaucucaca 60

auaccuucgu ucgacaggag gcucacaaca ggc 93

<210> 35
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 35
gggagacaag aauaaacgcu caauaccaaa cgcgcgguuu ucgucucgua auaaccaaau 60

gccucugagu ucgacaggag gcucacaaca ggc 93

<210> 36
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 36
gggagacaag aauaaacgcu caauaccaaa cgcgcaauuu ucaucuugua auaaccaaau 60

gccucugagu ucgacaggag gcucacaaca ggc 93

<210> 37
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 37
gggagacaag aauaaacgcu caacagucgc gaauuuuuua uucuuucuua caacaaagca 60

uagccucagu ucgacaggag gcucacaaca ggc 93

<210> 38
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 38
gggagacaag aauaaacgcu caagauugcg gauucucauc uuuccaacaa cgaacuagcc 60

ucuacuaguu cgacaggagg cucacaacag gc 92

<210> 39
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 39

gggagacaag aauaaacgcu caauugucaa cgaucgagca cguucucaca caaagccucu 60

uacuauaugu ucgacaggag gcucacaaca ggc 93

<210> 40
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 40
gggagacaag aauaaacgcu caacaaucgc guacguucuu gcguaacaaa cagccacugu 60

cauaaacguu cgacaggagg cucacaacag gc 92

<210> 41
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 41
gggagacaag aauaaacgcu caacguuuac gcguaaucuu guaauucaca uucucucaac 60

aagccuaguu cgacaggagg cucacaacag gc 92

<210> 42
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 42
gggagacaag aauaaacgcu caagacauca acaucucaac gaucuuguua cucucaacuc 60

aaauagcguu cgacaggagg cucacaacag gc 92

<210> 43
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 43
gggagacaag aauaaacgcu caaacguuac ucuugcaaca caaacuuuaa uagccucuua 60

uaguucguuc gacaggaggc ucacaacagg c 91

<210> 44
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 44
gggagacaag aauaaacgcu caaacguuac ucuugcaaca cccaaacuuu aauagccucu 60

uauaguucgu ucgacaggag gcucacaaca ggc 93

<210> 45
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 45
gggagacaag aauaaacgcu caaacguuac ucuugcaaca cccaaacuuu aauagccucu 60

uacagaaguu cgacaggagg cucacaacag gc 92

<210> 46
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 46
gggagacaag aauaaacgcu caauacagcg cuauucuucc aaccaaucau accaccuugu 60

cauguuaagu ucgacaggag gcucacaaca ggc 93

<210> 47
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 47
gggagacaag aauaaacgcu caacgaaucg aagcgauauu ccuuaccaau uaauuguaua 60

gccuuaguuc gacaggaggc ucacaacagg c 91

<210> 48
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 48
gggagacaag aauaaacgcu caauguugca acaucgaguc agcguguucu uccaagccuc 60

uauagaacgu ucgacaggag gcucacaaca ggc 93

<210> 49
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 49
gggagacaag aauaaacgcu caacaucgaa uacagccuuu aauccaaccu ccaauuucaa 60

ucgacuaagu ucgacaggag gcucacaaca ggc 93

<210> 50
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 50
gggagacaag aauaaacgcu caauucagcg auguucuaau caccacauaa caaacuauag 60

ccagaccugu ucgacaggag gcucacaaca ggc 93

<210> 51
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 51
gggagacaag aauaaacgcu caaugaucgu ugaauucaac uguccacuua acaaauuuca 60

gccacuaagu ucgacaggag gcucacaaca ggc 93

<210> 52
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 52
gggagacaag aauaaacgcu caauucgugu caacucaacc aaccaagccu ucugacguac 60

acuaagguuc gacaggaggc ucacaacagg c 91

<210> 53
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 53
gggagacaag aauaaacgcu caaacagcga uucgaucucu acccacaaca caaaugccuu 60

cacacauagu ucgacaggag gcucacaaca ggc 93

<210> 54
<211> 93
<212> RNA
<213> Artificial

<220>

<223> synthetic primers-aptamers

<400> 54
gggagacaag aauaaacgcu caaugcgcga auucuauccg uaugcaauuc augcauacau 60

uccaacuagu ucgacaggag gcucacaaca ggc 93

<210> 55
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 55
gggagacaag aauaaacgcu caauuagaau ucuaauuuga uaauauuacu ugccgccucc 60

acgaacacgu ucgacaggag gcucacaaca ggc 93

<210> 56
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 56
gggagacaag aauaaacgcu caaugauuuu gcagcacuuc uuguuaucuu aacgaacugu 60

ugaugaguuc gacaggaggc ucacaacagg c 91

<210> 57
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 57
gggagacaag aauaaacgcu caacuaagag guugacgcuu agcacuucca guaaccuaag 60

ccuucuaguu cgacaggagg cucacaacag gc 92

<210> 58
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 58
gggagacaag aauaaacgcu caauguuuga cuugauucuc uagcuuacaa auguuaacau 60

cugcaaaguu cgacaggagg cucacaacag gc 92

<210> 59
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 59
gggagacaag aauaaacgcu caaugucuug uuuauucgaa cucacauuaa caacaaugau 60

uagacggcgu ucgacaggag gcucacaaca ggc 93

<210> 60
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 60
gggagacaag aauaaacgcu caaccgcaac aagauugacg gcuugcguaa auucacaaga 60

uuucauuguu cgacaggagg cucacaacag gc 92

<210> 61
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 61
gggagacaag aauaaacgcu caacugugac gacaguuaag aucguauucu gccaccauac 60

cuguuguagu ucgacaggag gcucacaaca ggc 93

<210> 62
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 62
gggagacaag aauaaacgcu caauucacac acucaauuga acggugauuc aaguuauuag 60

cagccucagu ucgacaggag gcucacaaca ggc 93

<210> 63
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 63
gggagacaag aauaaacgcu caaacgcuug ucuuguuuuc gugagcuaaa guaucaguca 60

gaggcaauuc gacaggaggc ucacaacagg c 91

<210> 64
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 64
gggagacaag aauaaacgcu caaccguugu ucuacauguc acucaucacg cgagucuuuu 60

gucuacauuc gacaggaggc ucacaacagg c 91

<210> 65
<211> 89
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 65
gggagacaag aauaaacgcu caaccguugu ucuacauguc acucaucaua cgagucuuuu 60

gucuauucga caggaggcuc acaacaggc 89

<210> 66
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 66
gggagacaag aauaaacgcu caaccguugu ucuacauguc acucaucaug cgagucuuuu 60

gucuaauucg acaggaggcu cacaacaggc 90

<210> 67
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 67
gggagacaag aauaaacgcu caacgagacu uuaacguuug acuuguuuga ccaaaugugu 60

gauaccuucg acaggaggcu cacaacaggc 90

<210> 68
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 68
gggagacaag aauaaacgcu caagucaaau gggcguauua cguaaauuuu ccggcaguau 60

gugaagcauu cgacaggagg cucacaacag gc 92

<210> 69
<211> 90
<212> RNA

<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 69
gggagacaag aauaaacgcu caaugauuuu gcagcacuuc ucguuaucuu agcgagcugu 60

ugaugauucg acaggaggcu cacaacaggc 90

<210> 70
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 70
gggagacaag aauaaacgcu caaugauuuu gcagcacuuc uuguuaucuu aacgagcugu 60

ugaugguucg acaggaggcu cacaacaggc 90

<210> 71
<211> 89
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 71
gggagacaag aauaaacgcu caacgugcaa cgcacaaauu cuugaucauc ucaaugaugu 60

gugcuuucga caggaggcuc acaacaggc 89

<210> 72
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 72
gggagacaag aauaaacgcu caacgugcaa cgcacaaauu cuugaucauc ucaaugaugu 60

gugucuuucg acaggaggcu cacaacaggc 90

<210> 73
<211> 89
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 73
gggagacaag aauaaacgcu caacgugcga cauacaaauu cuugaucauc ccaaugaugu 60

gugcuuucga caggaggcuc acaacaggc 89

<210> 74
<211> 89

<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 74
gggagacaag aauaaacgcu caacgugcga cauacaaauu cuugaucauc ucaaugaugu 60

gugcuuucga caggaggcuc acaacaggc 89

<210> 75
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 75
gggagacaag aauaaacgcu caauaccaaa cgcgcaauuu ucaucuugua auaaccaaau 60

gccucugauu cgacaggagg cucacaacag gc 92

<210> 76
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 76
gggagacaag aauaaacgcu caauaccaaa cgcgcgauuu ucaucuugua auaaccaaau 60

gccucugauu cgacaggagg cucacaacag gc 92

<210> 77
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 77
gggagacaag aauaaacgcu caauugcauu uacucgaugu cccacaacaa ugugauaccu 60

cuuaugauuc gacaggaggc ucacaacagg c 91

<210> 78
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 78
gggagacaag aauaaacgcu caauugcauu uacucgaugu cccacgacaa ugugauaccu 60

cuuaugauuc gacaggaggc ucacaacagg c 91

<210> 79

<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 79
gggagacaag aauaaacgcu caauugcauu uacucgaugu cccacgacaa ugugauaccu 60

cuuaugguuc gacaggaggc ucacaacagg c 91

<210> 80
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 80
gggagacaag aauaaacgcu caauugcauu uacucgaugu uccacaacaa ugugauaccu 60

cuuaugauuc gacaggaggc ucacaacagg c 91

<210> 81
<211> 93
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 81
gggagacaag aauaaacgcu caaaacucug gggcgcuauu cucaucgcaa acccaaccgu 60

uguguaccuu ucgacaggag gcucacaaca ggc 93

<210> 82
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 82
gggagacaag aauaaacgcu caaacgugcg acauacaaau ucuugaucau cucaaugaug 60

ugugcuuucg acaggaggcu cacaacaggc 90

<210> 83
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 83
gggagacaag aauaaacgcu caagucguaa gguugcguau guguucgugu aaucucauug 60

cgagcucuuc gacaggaggc ucacaacagg c 91

<210> 84
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 84
gggagacaag aauaaacgcu caagucguaa gguuguguau guguucgugu aaucucauug 60

cgagcucuuc gacaggaggc ucacaacagg c 91

<210> 85
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 85
gggagacaag aauaaacgcu caaguugugc cauguuagcg cacaauuugu aauucaagag 60

cgcaaguucg acaggaggcu cacaacaggc 90

<210> 86
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 86
gggagacaag aauaaacgcu caaugccuac ucuugucauc ucuagagcca aauacaagcg 60

uuaacauucg acaggaggcu cacaacaggc 90

<210> 87
<211> 87
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 87
gggagacaag aauaaacgcu caaugguuga agcaugaguc guucuucuug ccaugugaaa 60

gcuuucgaca ggaggcucac aacaggc 87

<210> 88
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 88
gggagacaag aauaaacgcu caaugguugc aaaauacaug aacgucaauu uucagucuug 60

auaccuguuc gacaggaggc ucacaacagg c 91

<210> 89
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 89
gggagacaag aauaaacgcu caaaugccua cucuugucau cucugagcca aauacaagcg 60

uuaacauucg acaggaggcu cacaacaggc 90

<210> 90
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 90
gggagacaag aauaaacgcu caacgauuug uggcgacagg uuaaacgucg cuucaauuuc 60

gcagcauucg acaggaggcu cacaacaggc 90

<210> 91
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 91
gggagacaag aauaaacgcu caacgguaca ugcguugauu uucuugcaca cagccucuau 60

aacaacuuuc gacaggaggc ucacaacagg c 91

<210> 92
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 92
gggagacaag aauaaacgcu caaaugaauc ggaaagcgca aucuugaguu cuccuaccuu 60

uugugauucg acaggaggcu cacaacaggc 90

<210> 93
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 93
gggagacaag aauaaacgcu caacgacuug uaugucuuga ugugaaucuu cuaaucuacc 60

augagcauuc gacaggaggc ucacaacagg c 91

<210> 94
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 94
gggagacaag aauaaacgcu caagccucuc aacgauuaau guuucauuaa caugaucaau 60

cgccucaauu cgacaggagg cucacaacag gc 92

<210> 95
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 95
gggagacaag aauaaacgcu caaggucaaa aacguuugcu uguuuucagg auacaaugug 60

gagccauauu cgacaggagg cucacaacag gc 92

<210> 96
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 96
gggagacaag aauaaacgcu caauucagcg caacuguucg ucuuuccacg gcugugagac 60

uucagaauuc gacaggaggc ucacaacagg c 91

<210> 97
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 97
gggagacaag aauaaacgcu caauucagcg caacuguucg ucuuuccacg gcugugagac 60

uucaggauuc gacaggaggc ucacaacagg c 91

<210> 98
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 98
gggagacaag aauaaacgcu caauucagcg caacuguucg ucuuuccacg gcugugagac 60

uucggaauuc gacaggaggc ucacaacagg c 91

<210> 99
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 99
gggagacaag aauaaacgcu caauucagcg caacuguucg ucuuuccaug gcugugagac 60

uucagaauuc gacaggaggc ucacaacagg c 91

<210> 100
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 100
gggagacaag aauaaacgcu caauuuguug cgaaucgcac auauuggacg uucuguuugu 60

gugaguauuc gacaggaggc ucacaacagg c 91

<210> 101
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 101
gggagacaag aauaaacgcu caauuuguug cgaaucgcac guauuggacg uucuguuugu 60

gugaguauuc gacaggaggc ucacaacagg c 91

<210> 102
<211> 90
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 102
gggagacaag aauaaacgcu caauuuguug cgaauugcac auauuggacg uucuguugug 60

ugaguauucg acaggaggcu cacaacaggc 90

<210> 103
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 103
gggagacaag aauaaacgcu caagaacguu guauuuacuu gaccucucgc uaguuuagcu 60

uucuacauuc gacaggaggc ucacaacagg c 91

<210> 104
<211> 91
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 104
gggagacaag aauaaacgcu caauccauuu uggaugauug uugugauucu cguaauacaa 60

gccuucauuc gacaggaggc ucacaacagg c 91

<210> 105
<211> 92
<212> RNA
<213> Artificial

<220>
<223> synthetic primers-aptamers

<400> 105
gggagacaag aauaaacgcu caacgacacg uugccagccg gagccuuagu aacgugcuuu 60

gaugucgauu cgacaggagg cucacaacag gc 92

Claims

1. A method for selecting a nucleic acid aptamer specific for a protein selectively expressed on the cell surface of target cells comprising the steps of:

   a) incubating a collection of synthetic nucleic acid oligomers with control cells, allowing oligomers to bind to them;
   b) recovering a first set of unbound nucleic acid oligomers;
   c) incubating the first set of unbound nucleic acid oligomers with target cells, allowing the first set of unbound nucleic acid oligomers to bind to them;
   d) recovering nucleic acid oligomers bound to target cells;
   e) amplifying and sequencing the nucleic acid oligomers bound to target cells.

2. The method of claim 1 wherein the first set of unbound nucleic acid oligomers recovered in step b) is incubated with the control cells and a second set of unbound nucleic acid oligomers recovered in step b) is further processed as indicated in steps c), d) and e).

3. The method according to claim 1 or 2 wherein the collection of synthetic nucleic acid oligomers is a synthetic library.

4. The method according to any of claims 1 to 3 wherein the synthetic nucleic acid oligomers are labelled.

5. The method according to any of claims 1 to 4 wherein the nucleic acid oligomers are oligoribonucleotides or modified RNA-se resistant oligoribonucleotides.

6. The method according to any of claims 1 to 5 wherein the target cell is a tumor cell and the control cell is a tumor cell of the same cell type as the target cell but having a different phenotype.

7. The method according to claim 6 wherein the tumor cell is a glioma cell or a NSCLC cell.

8. The method according to claims 6 or 7 wherein the phenotype is selected from the group of: resistance to a given physical or chemical therapeutic drug, tumor mass growth properties, apoptosis, ability to metastasize or malignancy, drug treated tumor cell.

9. A nucleic acid aptamer obtainable according to the method of any of previous claims.

10. The nucleic acid aptamer according to claim 9 for medical use.

11. The nucleic acid aptamer according to claim 10 for the treatment of a tumor, also as targeting component for biocomplexes with nano particles or siRNAs.

12. The nucleic acid aptamer according to claim 10 for the diagnosis of a tumor and/or the follow-up of a therapy, also for molecular imaging.

13. The nucleic acid aptamer according to claim 10 for predicting a therapeutic response of a drug for a tumor.

14. The nucleic acid aptamer according to claims 11 to 13 wherein the tumor is a glioma or a NSCLC.

15. The nucleic acid aptamer according to claim 9 for the detection of a target cell.

16. The nucleic acid aptamer according to claim 15 wherein the target cell is a tumor cell.

17. The nucleic acid aptamer according to claim 16 wherein the tumor cell is a glioma cell or a NSCLC cell.

18. The nucleic acid aptamer according to claim 9 having a sequence selected from the group of: SEQ ID No.3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104 or 105.

19. A pharmaceutical composition comprising at least one nucleic acid aptamer according to claim 9 and suitable excipients and/or diluents and/or carrier.

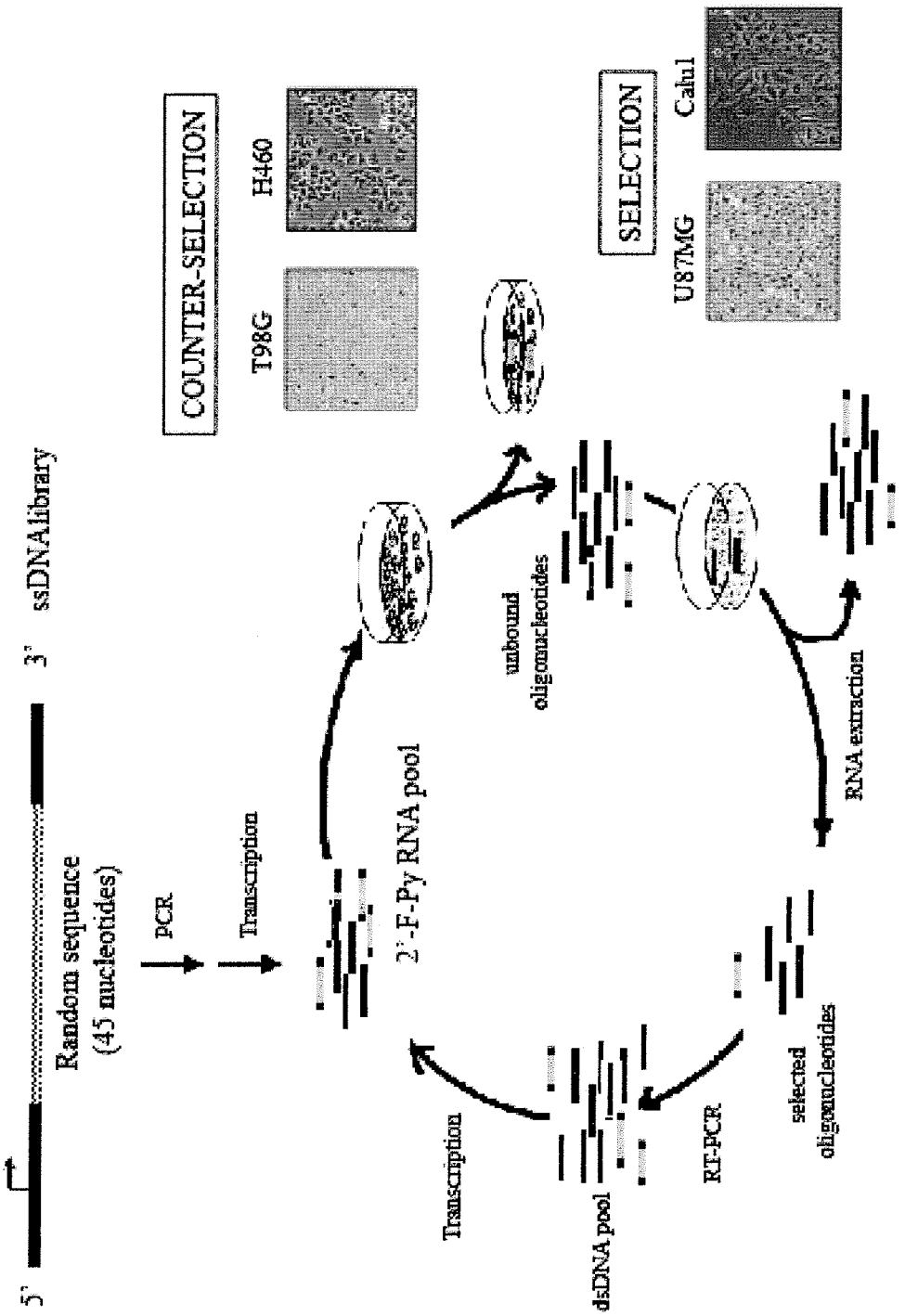

5'
3'
ssDNAlibrary
Random sequence
(45 nucleotides)
PCR
Transcription
2'-F-Py RNA pool
COUNTER-SELECTION
T98G
H460
unbound
oligonucleotides
SELECTION
U87MG
Cal51
RNA extraction
selected
oligonucleotides
RT-PCR
dsDNA pool
Transcription

Fig 1

A

SELEX round	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SELEX condition (Glioma cell as target)														
Cell number ($x10^6$)	10	10	10	10	10	10	10	10	10	10	10	10	10	10
RNA amount (x100pmol)	5	8	8	3	3	3	3	3	3	3	3	3	3	3
Incubation time (min)	30	30	30	30	30	30	30	30	30	30	15	15	15	15
Counter-selection number	1	1	1	1	1	1	1	1	1	1	2	2	2	2
Whashing number	1	2	3	3	4	4	5	5	5	5	5	5	5	5
SELEX condition (NSCLC as target)														
Cell number ($x10^6$)	2	2	2	2	2	2	2	2	2	2	2	2	2	2
RNA amount (x100pmol)	8	8	8	8	8	8	8	8	7	7	7	8	7	7
Incubation time (min)	30	30	30	30	30	15	15	15	15	15	15	15	15	15
Counter-selection number	1	1	1	1	2	2	2	2	2	2	2	2	2	2
Whashing number	3	3	3	3	3	3	3	3	3	5	5	5	5	5
Poly(I (10ng/μl)	-	-	-	-	-	-	-	-	-	-	-	-	+	+

B

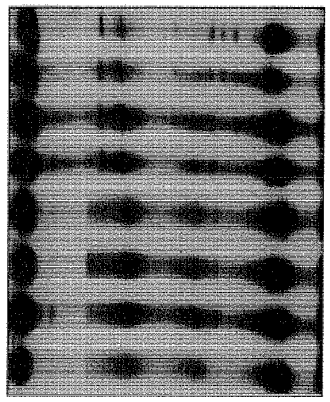


Fig 2

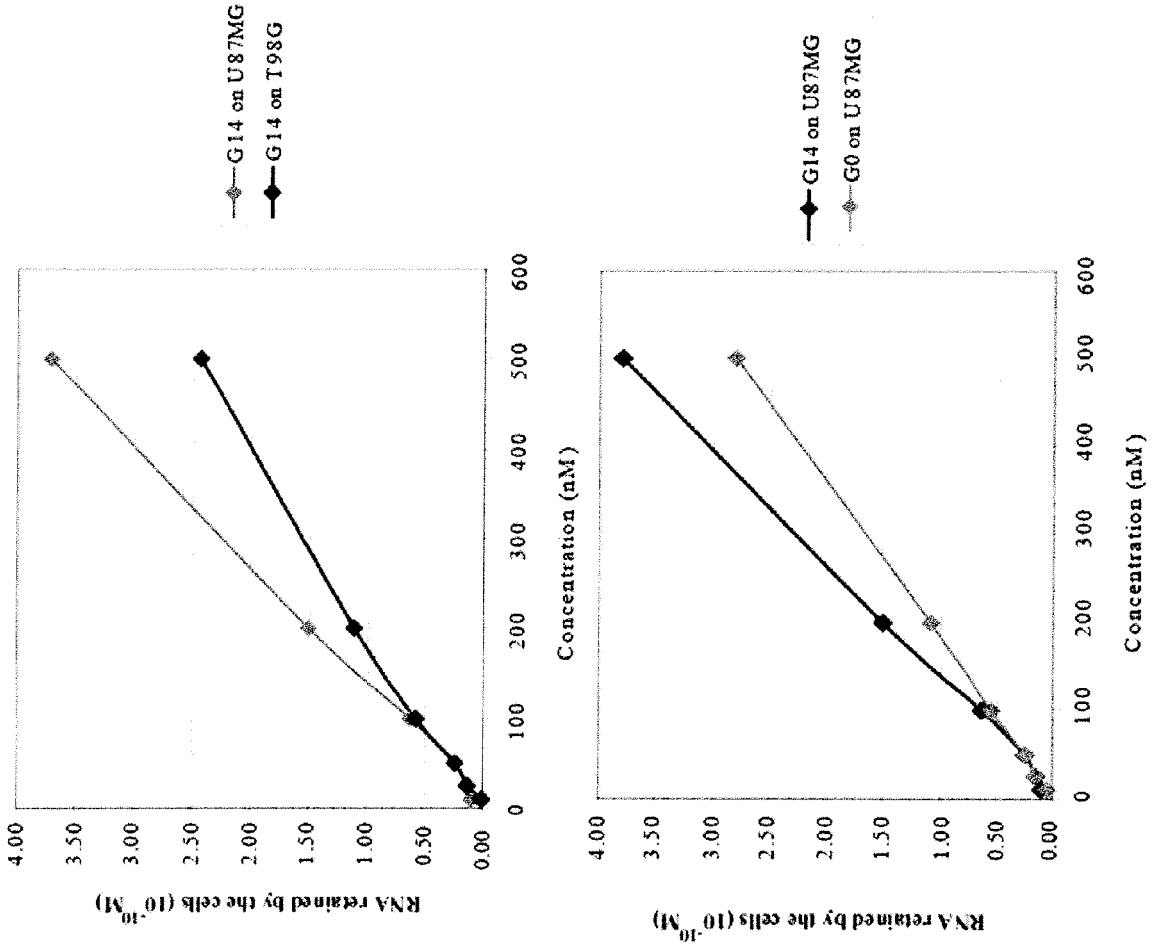

G14 on U87MG
G14 on T98G
RNA retained by the cells (10-10M)
Concentration (nM)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
100
200
300
400
500
600
G14 on U87MG
G0 on U87MG
RNA retained by the cells (10-10M)
Concentration (nM)

Fig 3

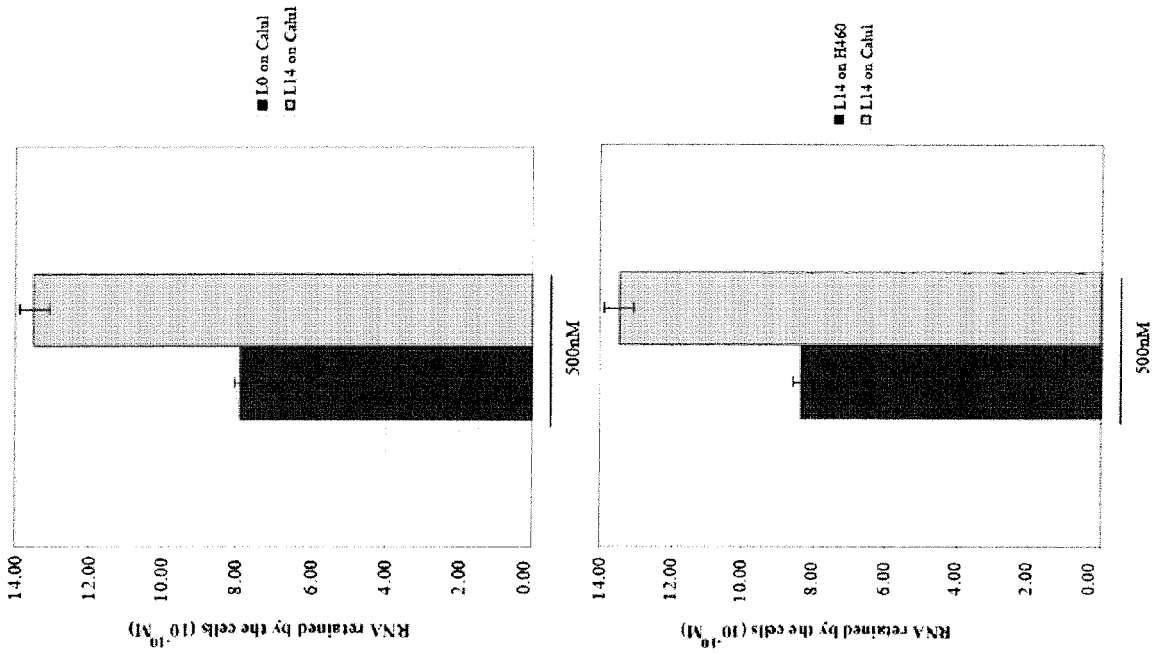

L0 on Calu1
L14 on Calu1
L14 on H460
L14 on Calu1
500nM
500nM
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
RNA retained by the cells (10-10M)
RNA retained by the cells (10-10M)

Fig 4


10
20
30
40
50
60
B20-
D7-
C4-
C20-
A2A21-
C15-
C24-
C8-
D14-
D6-
B10-
A7-
B2-
B19-
A6B15-
C2-
C10-
C1-
B22-
C5-
D23-
C22-
D2-
B13-
B3-
C9-
A20-
A2-
D3-
C16-
C7-
C12-
D9-
A9-
D21-
C18-
C23-
C6-
D13-
A4-
A5-
A10C13-
D18-
D5-
C14-
D19-
D4-
B7-
B8-
D22-
C3C11D10-
B17-
B14-
A3A1B4B8C19D11-
B16-
B4-
D12-
C21-
D15-
D1D20-

Fig 5

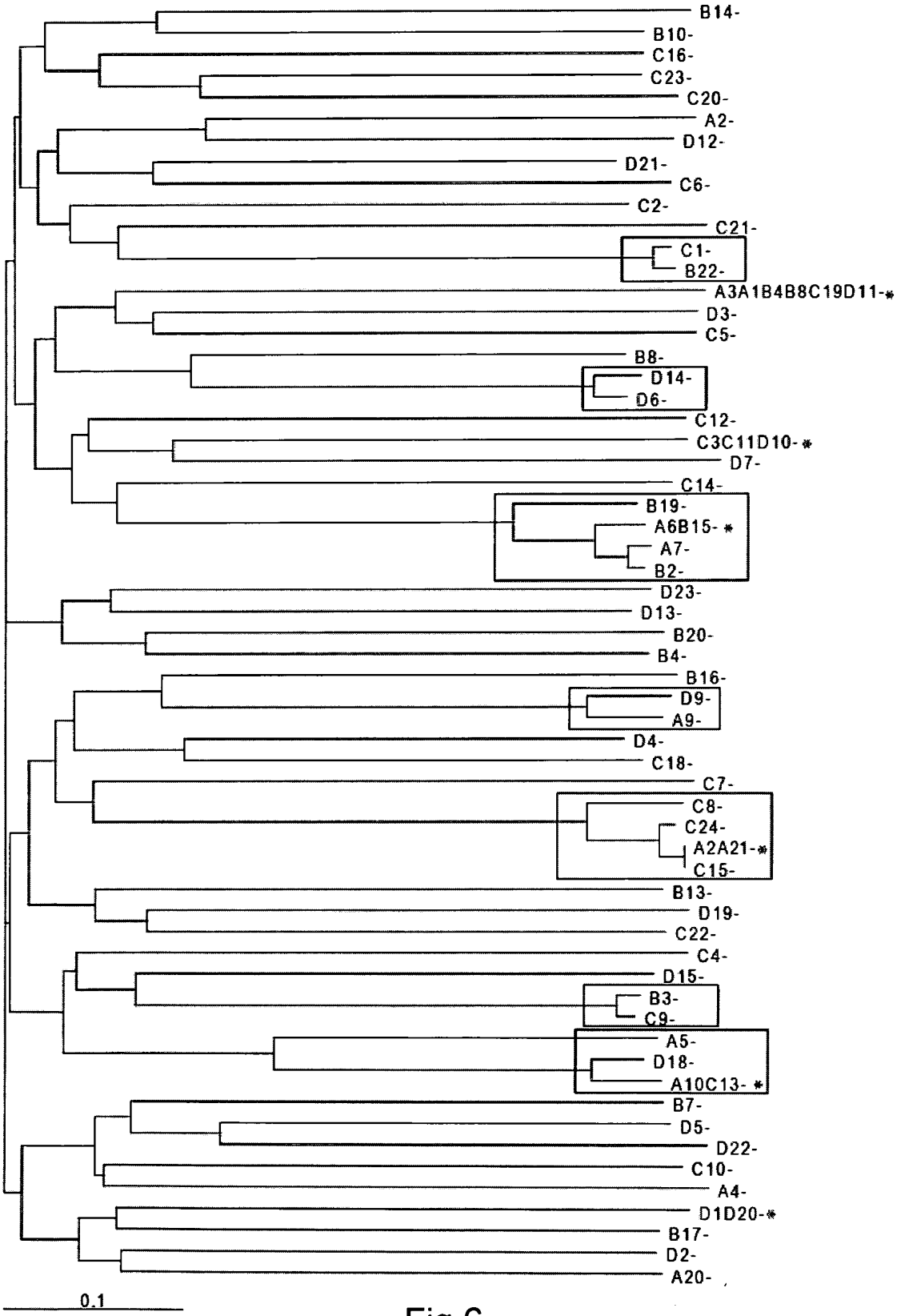

B14-
B10-
C16-
C23-
C20-
A2-
D12-
D21-
C6-
C2-
C21-
C1-
B22-
A3A1B4B8C19D11-*
D3-
C5-
B8-
D14-
D6-
C12-
C3C11D10-*
D7-
C14-
B19-
A6B15-*
A7-
B2-
D23-
D13-
B20-
B4-
B16-
D9-
A9-
D4-
C18-
C7-
C8-
C24-
A2A21-*
C15-
B13-
D19-
C22-
C4-
D15-
B3-
C9-
A5-
D18-
A10C13-*
B7-
D5-
D22-
C10-
A4-
D1D20-*
B17-
D2-
A20-
0.1

Fig 6

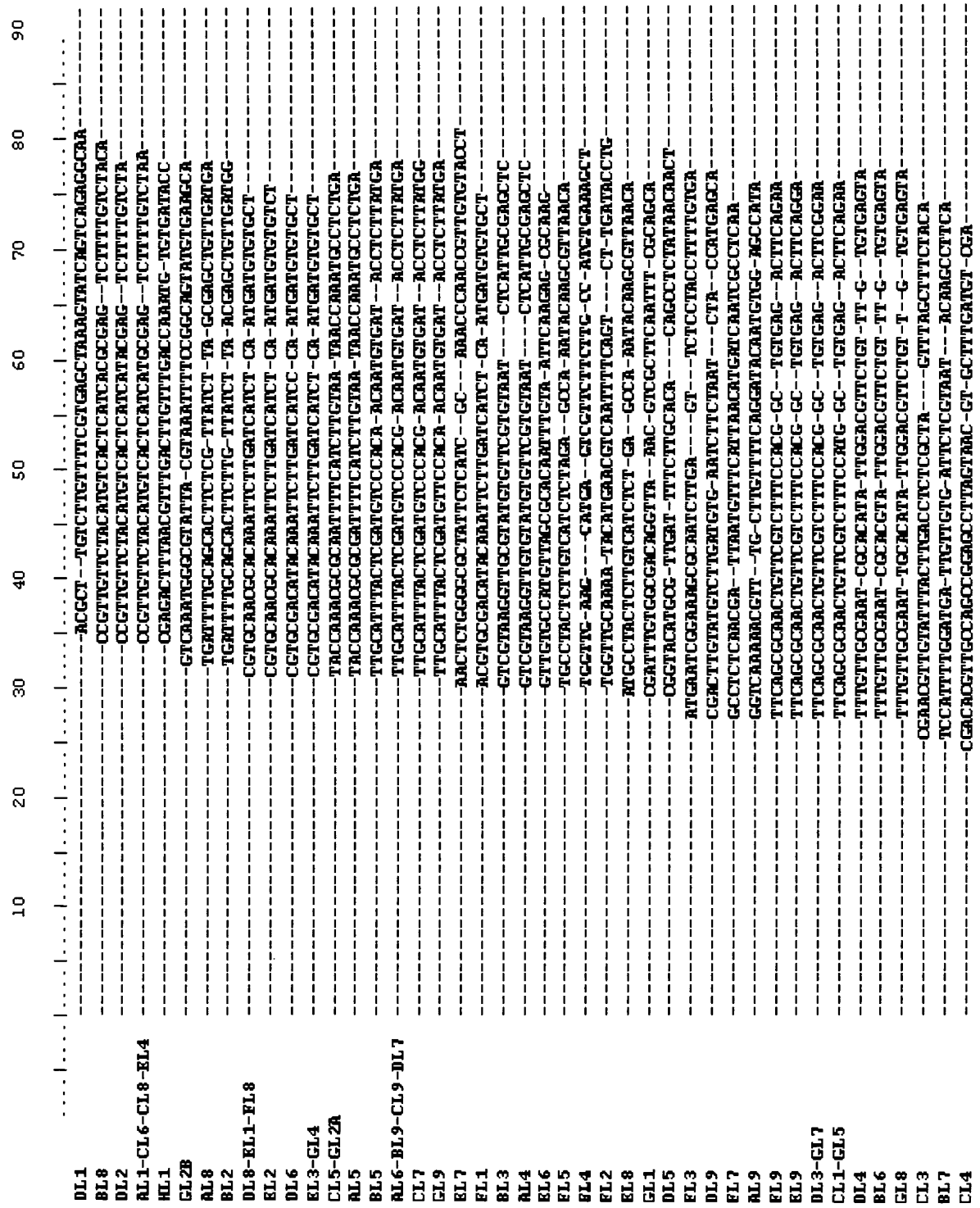


Fig 7

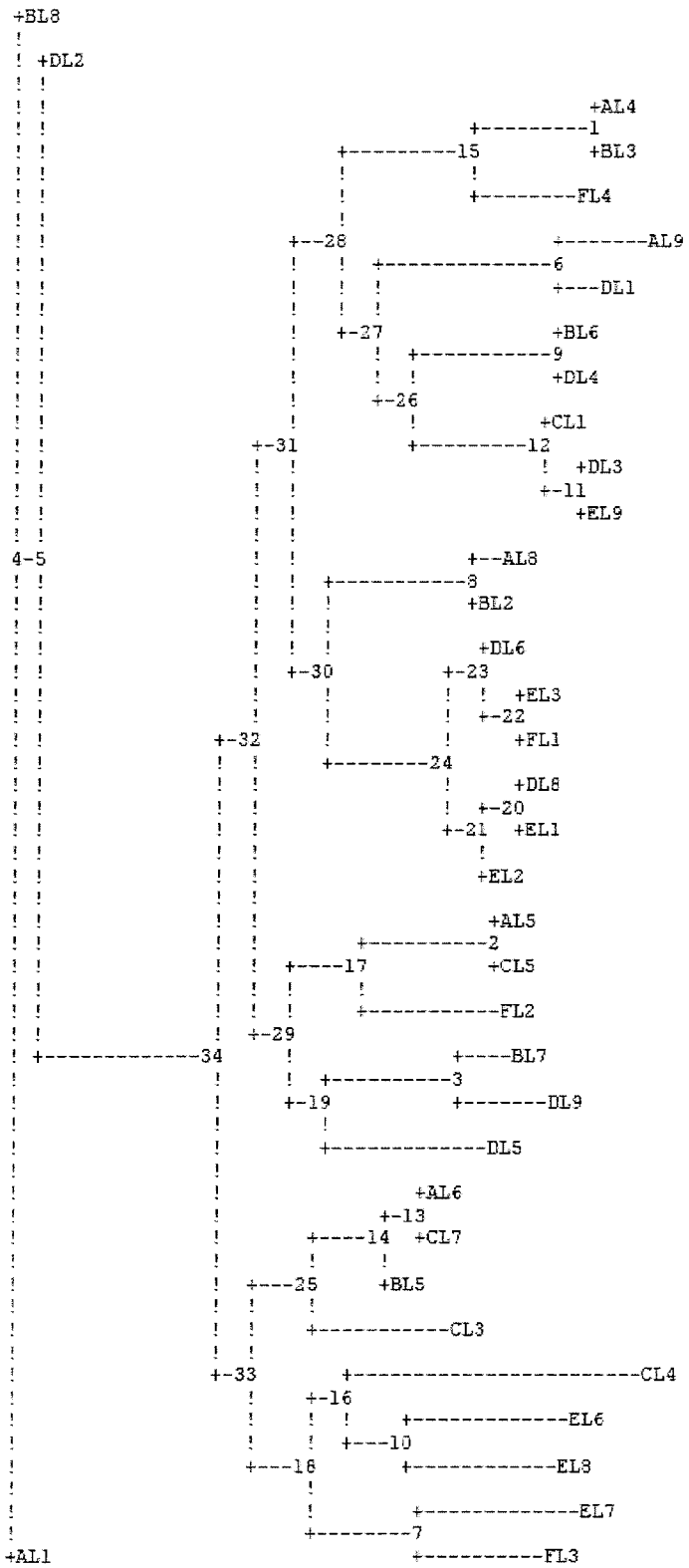

+BL8
+DL2
+AL4
1
15
+BL3
FL4
28
AL9
6
DL1
27
BL6
9
DL4
26
CL1
31
12
DL3
11
EL9
4-5
AL8
8
BL2
DL6
30
23
EL3
22
32
FL1
24
DL8
20
21
EL1
EL2
AL5
2
17
CL5
FL2
29
34
BL7
3
19
DL9
DL5
AL6
13
14
CL7
25
BL5
CL3
33
CL4
16
EL6
10
18
EL8
EL7
7
AL1
FL3

Fig 8

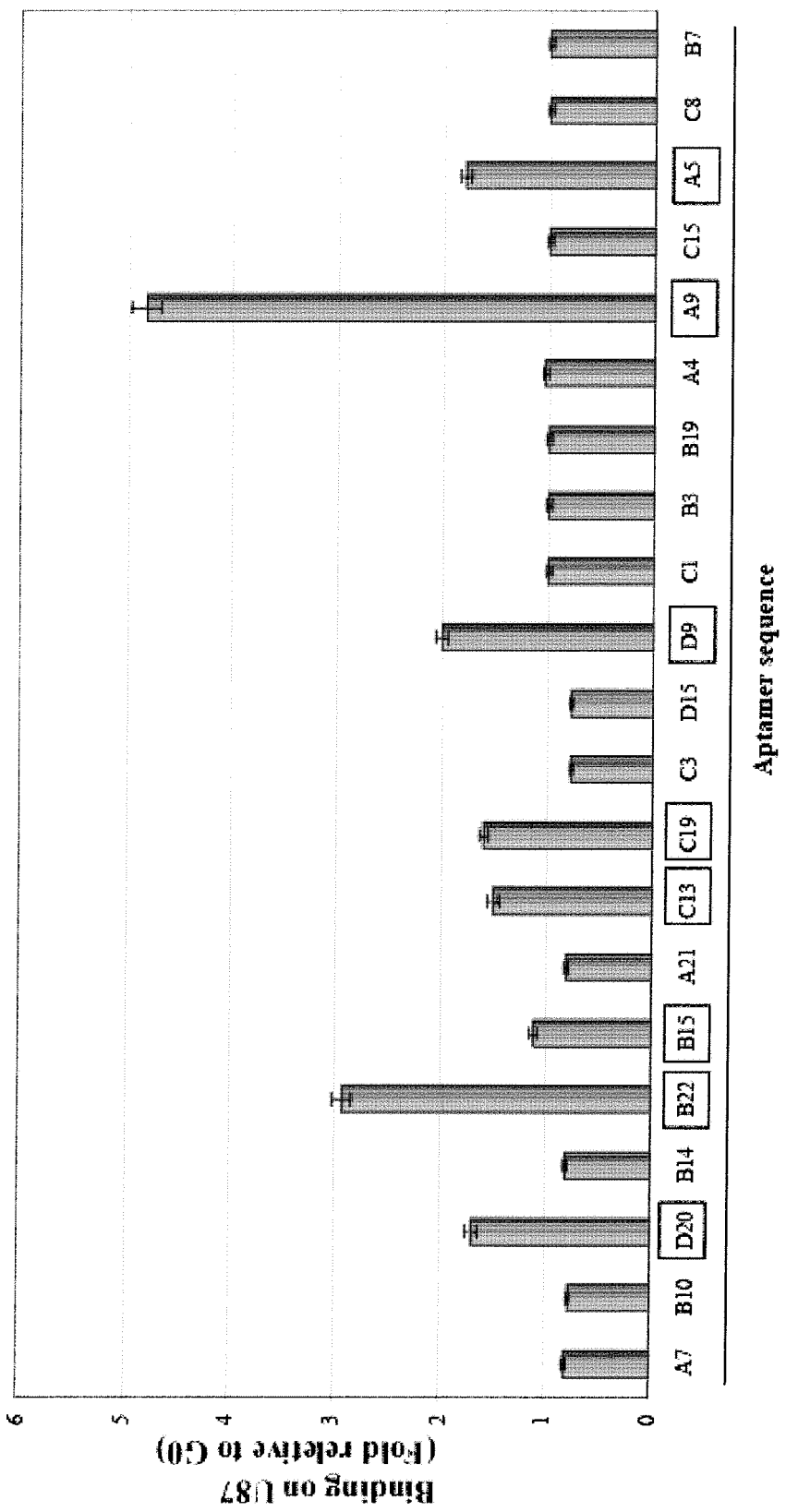
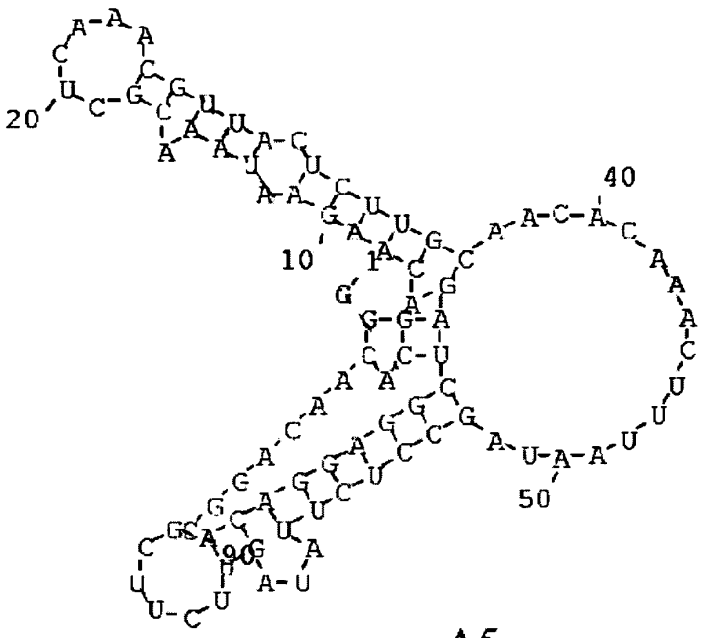
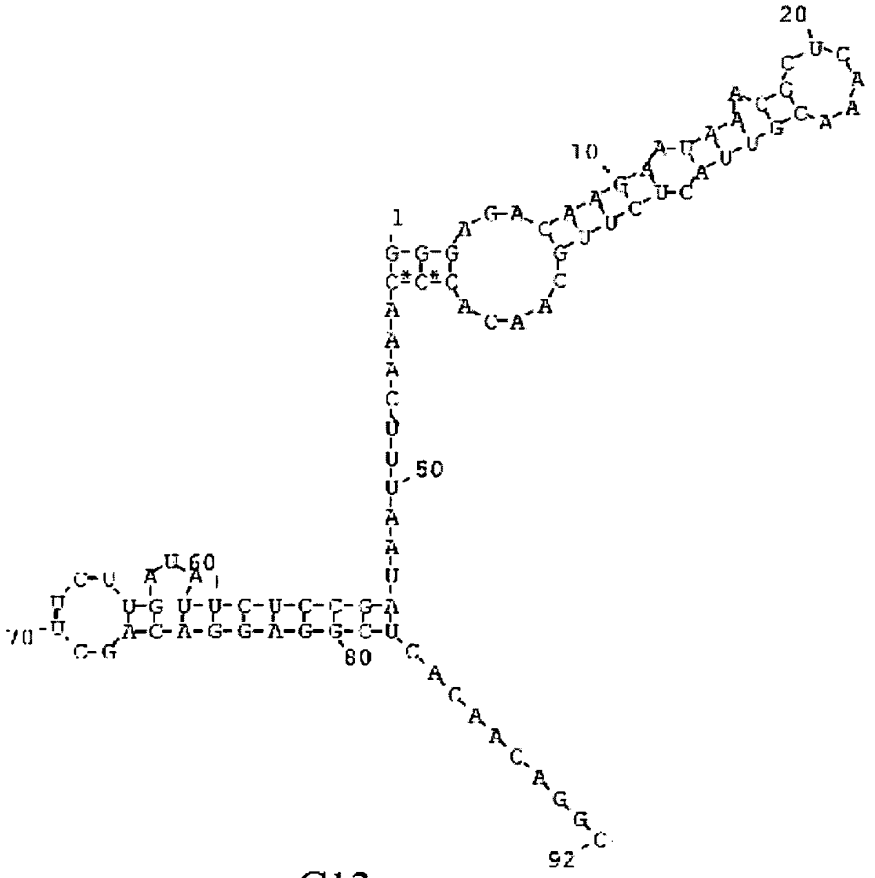

Binding on U87
(Fold relative to G0)
0
1
2
3
4
5
6
A7
B10
D20
B14
B22
B15
A21
C13
C19
C3
D15
D9
C1
B3
B19
A4
A9
C15
A5
C8
B7
Aptamer sequence

Fig 9

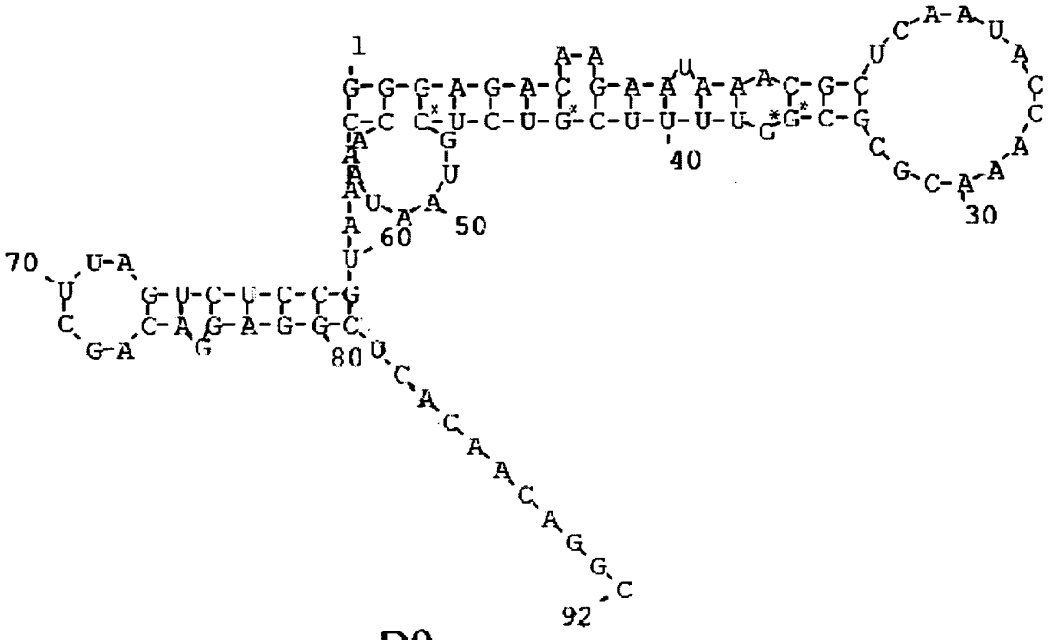


A5

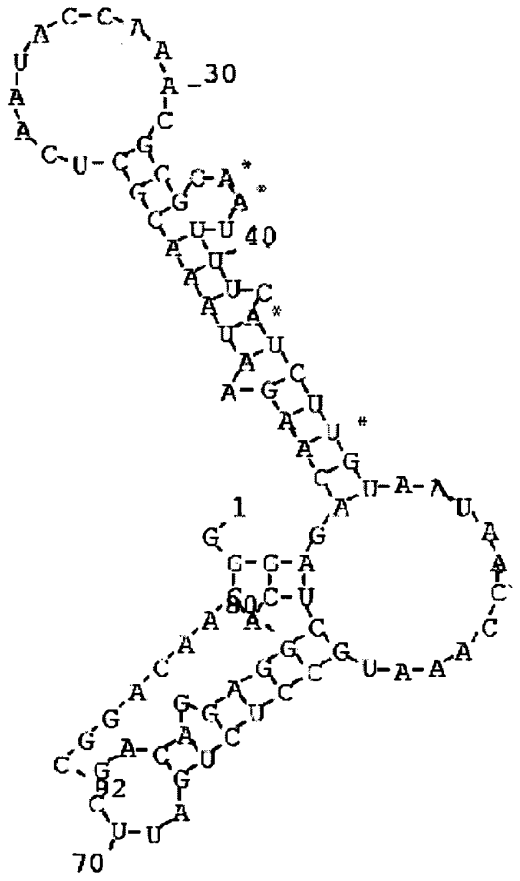


C13

Fig 10



D9



A9

Fig 10

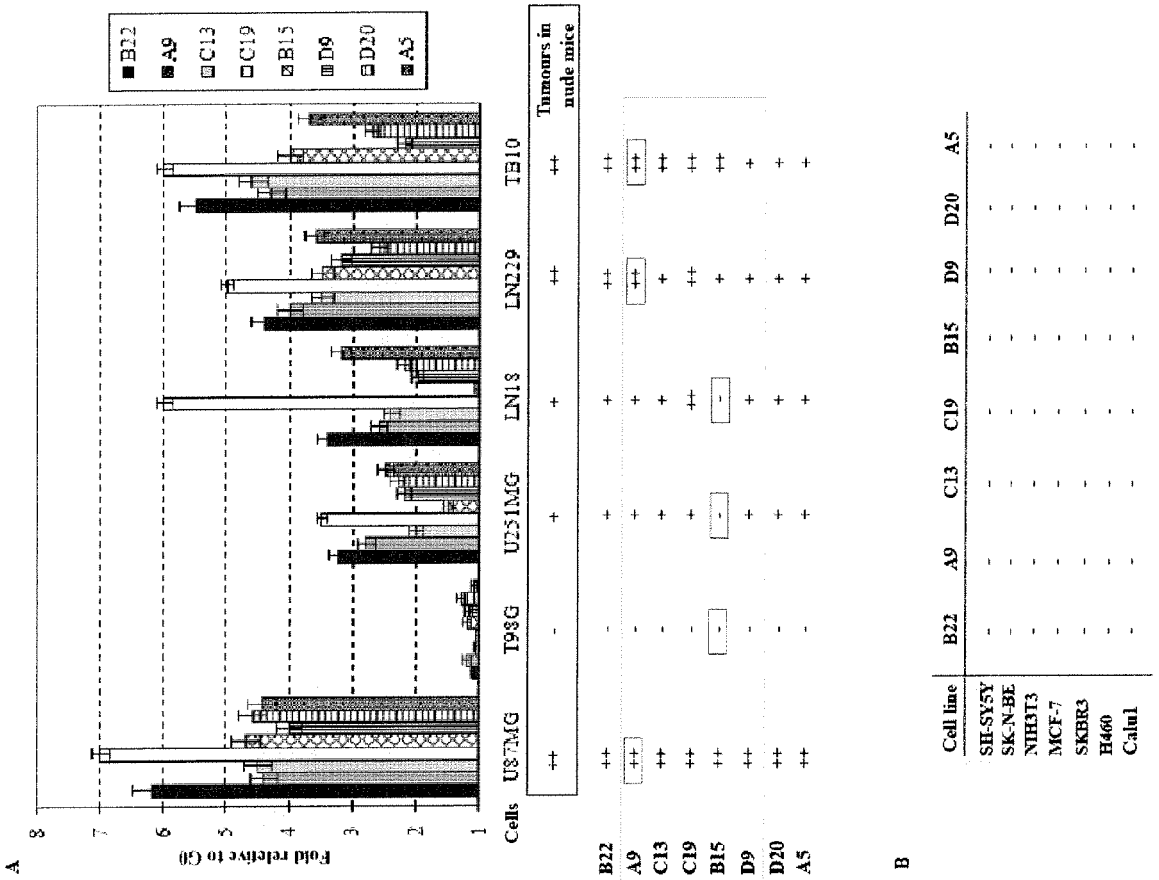
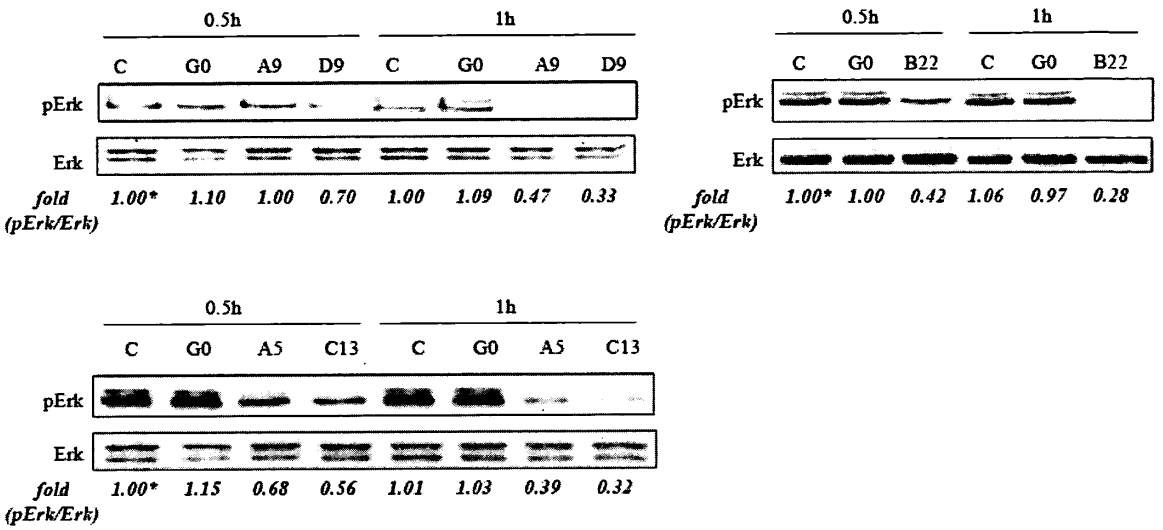

A
B22
A9
C13
C19
B15
D9
D20
A5
Fold reduction to G0
8
7
6
5
4
3
2
1
Cells
U87MG
T98G
U251MG
LN18
LN229
T810
Tumours in nude mice
B22
A9
C13
C19
B15
D9
D20
A5
B
Cell line
SH-SY5Y
SK-N-BE
NIH3T3
MCF-7
SKBR3
H460
Calu1

Fig 11

A


0.5h
1h
C G0 A9 D9 C G0 A9 D9
pErk
Erk
fold (pErk/Erk) 1.00* 1.10 1.00 0.70 1.00 1.09 0.47 0.33
0.5h
1h
C G0 B22 C G0 B22
pErk
Erk
fold (pErk/Erk) 1.00* 1.00 0.42 1.06 0.97 0.28
0.5h
1h
C G0 A5 C13 C G0 A5 C13
pErk
Erk
fold (pErk/Erk) 1.00* 1.15 0.68 0.56 1.01 1.03 0.39 0.32

B

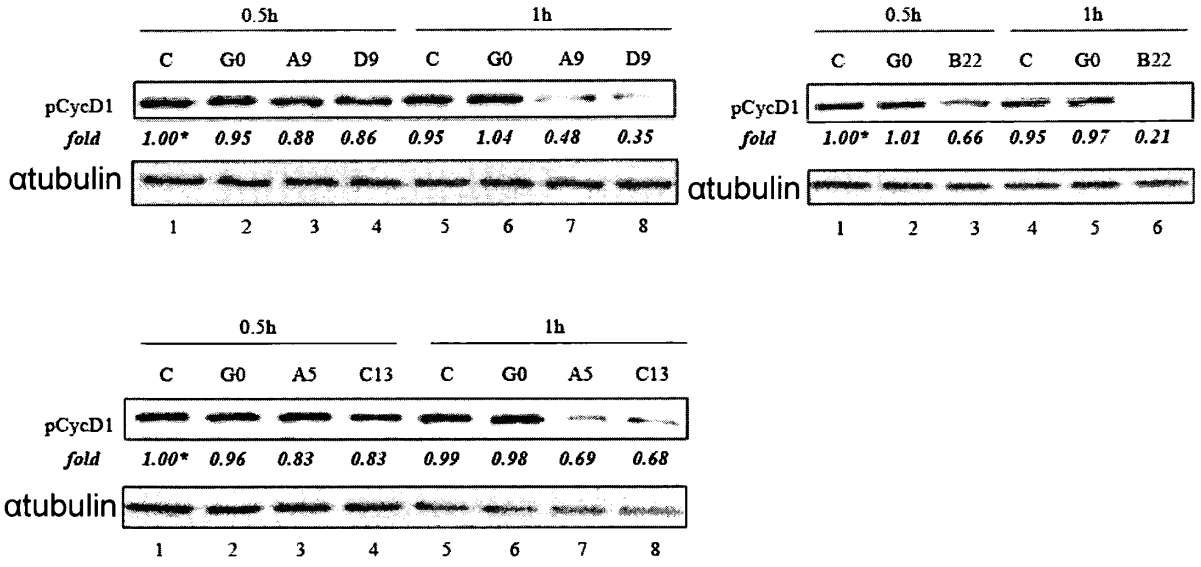

0.5h
1h
C G0 A9 D9 C G0 A9 D9
pCycD1
fold 1.00* 0.95 0.88 0.86 0.95 1.04 0.48 0.35
atubulin
1 2 3 4 5 6 7 8
0.5h
1h
C G0 B22 C G0 B22
pCycD1
fold 1.00* 1.01 0.66 0.95 0.97 0.21
atubulin
1 2 3 4 5 6
0.5h
1h
C G0 A5 C13 C G0 A5 C13
pCycD1
fold 1.00* 0.96 0.83 0.83 0.99 0.98 0.69 0.68
atubulin
1 2 3 4 5 6 7 8

Fig 13

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 08 10 5194

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	WO 2008/019142 A (MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US]) 14 February 2008 (2008-02-14) * page 11 - page 14 * * the whole document * * page 27 - page 28 *	1-5,7, 9-12, 14-17,19	INV. C12N15/11 A61K31/7088
X	WO 2005/093097 A (COMMISSARIAT A L'ENERGIE ATOMIQUE [FR]) 6 October 2005 (2005-10-06) * the whole document *	1-6, 9-13,15, 16,19	
X	PESTOURIE C. ET AL.: "Comparison of different strategies to select aptamers against a transmembrane protein target" OLIGONUCLEOTIDES, vol. 16, no. 4, 2006, pages 323-335, XP002514645 * the whole document *	1-3,5,6, 9,15,16	
X,D	CERCHIA L. ET AL.: "Neutralizing aptamers from whole-cell SELEX inhibit the RET Receptor Tyrosine Kinase" PLOS BIOLOGY, vol. 3, no. 4, April 2005 (2005-04), pages 697-704, XP002514646 * the whole document *	1-3,5,6, 9,15,16	TECHNICAL FIELDS SEARCHED (IPC) C12N
X	WO 2006/096754 A (ARCHEMIX CORP [US];) 14 September 2006 (2006-09-14) * page 10 - page 14 * * the whole document *	1-5, 9-13,15, 16,19	

-/--

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
The Hague	11 February 2009	Macchia, Giovanni

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

4

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 08 10 5194

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	WANG CHENGLONG ET AL.: "Single-stranded DNA aptamers that bind differentiated but not parental cells: subtractive systematic evolution of ligands by exponential enrichment" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 102, no. 1, 10 April 2003 (2003-04-10), pages 15-22, XP002484057 ISSN: 0168-1656 * the whole document *	1-4,6,9, 15,16	
X	BLANK M. ET AL.: "Systematic evolution of a DNA aptamer binding to rat brain tumor microvessels" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOCHEMICAL BIOLOGISTS, BIRMINGHAM,; US, vol. 276, no. 19, 11 May 2001 (2001-05-11), pages 16464-16468, XP008067444 ISSN: 0021-9258 * the whole document *	1-4,9, 12,15	TECHNICAL FIELDS SEARCHED (IPC)
A	CHEN C.-K. ET AL.: "Subtractive SELEX against two heterogeneous target samples: Numerical simulations and analysis" COMPUTERS IN BIOLOGY AND MEDICINE, NEW YORK, NY, US, vol. 37, no. 6, June 2007 (2007-06), pages 750-759, XP022016727 ISSN: 0010-4825 * page 752; figure 1 * * the whole document *		

-/--

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
The Hague	11 February 2009	Macchia, Giovanni

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

4

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 08 10 5194

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	WO 96/34874 A (NEXSTAR PHARMACEUTICALS, INC. [US]; SCHERING AG [DE]) 7 November 1996 (1996-11-07) * page 5 - page 16 * * the whole document *		
A	XUE-CHAI CHEN ET AL.: "Quantum dot-labeled aptamer nanoprobes specifically targeting glioma cells" NANOTECHNOLOGY, IOP, BRISTOL, GB, vol. 19, no. 23, 11 June 2008 (2008-06-11), page 235105, XP020136589 ISSN: 0957-4484 * the whole document *		
A	CERCHIA L. AND DE FRANCISCIS V.: "Noncoding RNAs in cancer medicine" JOURNAL OF BIOMEDICINE & BIOTECHNOLOGY, [Online] 2006, pages 1-8, XP002514647 Retrieved from the Internet: URL:http://www.hindawi.com/GetArticle.aspx?doi=10.1155/JBB/2006/73104> [retrieved on 2009-02-11] Article No. 73104 * the whole document *		TECHNICAL FIELDS SEARCHED (IPC)
A	CERCHIA L. ET AL.: "Nucleic acid aptamers in cancer medicine" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 528, no. 1-3, 25 September 2002 (2002-09-25), pages 12-16, XP004383225 ISSN: 0014-5793 * the whole document *		
	-/--		

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
The Hague	11 February 2009	Macchia, Giovanni

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

4

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 08 10 5194

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	PESTOURIE C. ET AL.: "Selection d'aptameres pour l'imagerie moleculaire" MEDECINE NUCLEAIRE, ELSEVIER, PARIS, FR, vol. 31, no. 9, 1 September 2007 (2007-09-01), page 485, XP022376729 ISSN: 0928-1258 * the whole document * -----		

TECHNICAL FIELDS SEARCHED (IPC)

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
The Hague	11 February 2009	Macchia, Giovanni

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

4

EPO FORM 1503 03.82 (P04C01)

Europäisches Patentamt
European Patent Office
Office européen des brevets

Application Number
EP 08 10 5194

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing claims for which payment was due.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

see annex

☐ The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).

Europäisches Patentamt
European Patent Office
Office européen des brevets

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 08 10 5194

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-8 all totally; 9-19 all partially

A method for selecting a nucleic acid aptamer, according to claims 1-8.
A nucleic acid aptamer obtainable according to the method of any of claims 1-8, in particular related to glioma.

1.1. claims: 1-8

A method for selecting a nucleic acid aptamer, according to claims 1-8.

1.2. claims: 9-19 all partially

A nucleic acid aptamer obtainable according to the method of any of claims 1-8, in particular related to glioma.

---

2. claims: 9-19 all partially

A nucleic acid aptamer obtainable according to the method of any of claims 1-8, in particular related to NSCLC.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 08 10 5194

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

11-02-2009

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008019142 A	14-02-2008	NONE	
WO 2005093097 A	06-10-2005	CA 2559871 A1	06-10-2005
		EP 1756305 A1	28-02-2007
		FR 2867784 A1	23-09-2005
		JP 2007529213 T	25-10-2007
		US 2008227735 A1	18-09-2008
WO 2006096754 A	14-09-2006	AU 2006220621 A1	14-09-2006
		CA 2600418 A1	14-09-2006
		EP 1863828 A2	12-12-2007
		JP 2008536485 T	11-09-2008
		US 2009105172 A1	23-04-2009
WO 9634874 A	07-11-1996	AT 401424 T	15-08-2008
		AT 276266 T	15-10-2004
		AU 727460 B2	14-12-2000
		AU 5720096 A	21-11-1996
		CA 2219106 A1	07-11-1996
		CA 2641149 A1	07-11-1996
		DE 69633386 D1	21-10-2004
		DE 69633386 T2	22-09-2005
		DK 1564290 T3	17-11-2008
		EP 1564290 A2	17-08-2005
		EP 2000534 A2	10-12-2008
		EP 0824540 A1	25-02-1998
		ES 2310695 T3	16-01-2009
		JP 11504812 T	11-05-1999

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5580737 A **[0005]**
- WO 2007142713 A **[0006]**

Non-patent literature cited in the description

- **Tuerk C. ; Gold L.** *Science,* 1990, vol. 249, 505-510 **[0065]**
- **Ellington ; Szostak.** *Nature,* 1990, vol. 346, 818-22 **[0065]**
- **Green LS et al.** *Chem Biol.,* 1995, vol. 2 (10), 683-95 **[0065]**
- **Tasset DM ; Kubik MF ; Steiner J.** *Mol. Biol.,* 1997, vol. 272 (5), 688-98 **[0065]**
- **Ruckman J et al.** *J. Biol. Chem.,* 1998, vol. 273 (32), 20556-67 **[0065]**
- **Cerchia et al.** *PLoS Biol.,* 2005, vol. 3 (4), e123 **[0065]**
- **Zuker, M.** *Nucleic Acids Res.,* 2003, vol. 31, 3406-3415 **[0065]**
- **Zanca C. et al.** *J Cell Mol. Med.,* 15 February 2008 **[0065]**
- **Esposito CL et al.** *PLoS ONE,* 2008, vol. 3 (2), e1643 **[0065]**
- **Buckley MF et al.** *Oncogene,* 1993, vol. 8 (8), 2127-33 **[0065]**
- **Cerchia L et al.** *Biochem J.,* 2003, vol. 372, 897-903 **[0065]**
- **Ishii N et al.** *Brain Pathol.,* 1999, vol. 9, 469-79 **[0065]**
- **Nishikawa R et al.** *Proc Nati Acad Sci.,* 1994, vol. 91, 7727-31 **[0065]**
- **Pallini R et al.** *Int. J. Cancer,* 2006, vol. 118, 2158-67 **[0065]**